University of Alberta

Development of a combined sex pheromone-based monitoring system to detect population density changes and monitor moth condition of *Malacosoma disstria* and *Choristoneura conflictana*

by

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In memory of my father, Don Jones, who showed me the easy pleasure of a walk in the woods.

ABSTRACT

A combined sex pheromone-based monitoring tool was developed for the aspen defoliators *Malacosoma disstria* and *Choristoneura conflictana* in Alberta, Canada. A system employing a single synthetic pheromone blend formulated in one lure to monitor two forest pests simultaneously represents a novel approach. The combined lure attracted both *M. disstria* and *C. conflictana* males throughout their flight period but lure age had a negative effect on attraction of *M. disstria*. Male moth captures were related to abundance of immature stages both within and between generations for each species but were not predictive of immature counts in the subsequent generation for either species. The combined lure does not attract *M. disstria* males in direct proportion to population density. Captured males of each species were measured for wing area and pathogen load as indicators of moth condition. The application of pheromone trapping for ecological studies is discussed.

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Chapter 1

Introduction

Background

Many species of forest Lepidoptera exhibit population cycles with large increases in abundance over a relatively short period of time. At least 18 species of forest Lepidoptera in North America and Europe reach outbreak population densities every 8-10 years (Myers, 1988) but causes of outbreaks are largely unknown (Berryman, 1996). The forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae) and large aspen tortrix, *Choristoneura conflictana* (Walker) (Lepidoptera: Tortricidae) are both outbreaking species and serious defoliators of trembling aspen, *Populus tremuloides* Michx. (Salicaceae) in Alberta, Canada. Repeated defoliation of aspen by *M. disstria* is associated with reduced growth (Hildahl & Reeks, 1960; Churchill et al., 1964) and even tree mortality (Candau et al., 2002) while defoliation from less severe *C. conflictana* outbreaks may cause reduced tree vigor and reduced incremental growth (Ives & Wong, 1988; Cerezke, 1992). Trembling aspen is increasing in commercial value (Hogg *et al.*, 2002; Brandt *et al.*, 2003) thus tools to monitor defoliator populations and warn of incipient outbreaks are needed in managed aspen forests. Sex pheromone-based monitoring of adult insect populations is an essential element in successful pest management programs (Baker & Heath, 2005). *M. dissstria* and *C. conflictana* are sympatric aspen defoliators with overlapping adult flight times. The species are distantly related, do not share sex pheromone components, and adults are morphologically distinct. For these reasons, this system is an ideal candidate for taking a novel approach to sex pheromone-based monitoring by combining pheromones for both species in a single formulation.

Moth pheromone biology

Female moths send a specific chemical signal, a sex pheromone, over long distances to attract conspecific males. Most female moths produce and emit a blend of fatty acid-derived molecules that consist of 10- to 18- carbon straight chain compounds with alcohol, acetate, or aldehyde functional groups (Ando *et al.*, 2004) and function as a unit over long distances (Baker & Heath, 2005). A second major class of moth sex pheromones comprised of polyene hydrocarbons and epoxides has been identified from species within the large Noctuoidea and Geometroidea groups (Millar, 2000). Moth sex pheromones are often multi-component blends with one major component and several minor components that occur in lesser quantities. Species specificity of sex pheromones is achieved through differing structures and ratios of components, and the presence of behavioral antagonists that repel heterospecific males (Cardé & Haynes, 2004). Other strategies may be employed simultaneously to achieve distinct communication channels such as differences in timing and spacing of mating behaviour, as well as male-produced courtship pheromones (Cardé & Haynes, 2004).

Emission of pheromone from a female moth results in a heterogeneous plume of odour without definite boundaries. The odour plume consists of filamentous strands of pheromone interspersed with clean air pockets (Baker & Heath, 2005). The primary olfactory organ of the male moth is the antenna. Sensillae on the antennae house receptor neurons that pheromone molecules enter to elicit a signal that is propagated to the central nervous system (Hansson, 1995). Upon responding to the odour, male moths fly upwind in a zigzag pattern across the plume to locate the source. Male moths react to subtle

changes in flux between pheromone strand concentration and clean air pockets while flying upwind to locate the pheromone source (Mafro-Neto & Cardé, 1994; Vickers & Baker, 1994).

Pheromone-based monitoring

The reliance of moths on chemical signaling for mate finding makes sex pheromone communication an avenue of exploitation for Integrated Pest Managment (IPM). Synthetic pheromones used in IPM programs serve to attract and capture male moths for population assessment or control. The most widespread use of sex pheromones in pest management, since their discovery almost 50 years ago is for monitoring populations of insect pest species (Baker & Heath, 2005). Pheromone-baited traps are effective tools in IPM because they are easy to operate, species-specific, and effective at low population densities (Elkinton & Cardé, 1981). Pheromone-baited traps may be used to determine the presence/absence of a pest species, to provide estimates of population density, or to monitor a population and identify a threshold density to time management strategies (Howse *et al.*, 1998). Pheromone trapping is particularly suited for monitoring forest Lepidoptera that experience periodic irruptions in population density. The increase phase of a population cycle often occurs gradually, thus incipient outbreaks may be detected with pheromone traps. For a pheromone-based monitoring system to predict outbreaks, trap catch must closely correlate with other estimates of population density (Sanders, 1988).

Relationships between pheromone trap catch and population density estimates of immature stages have been established for several species of forest Lepidoptera.

Pheromone-based monitoring has proven to be an effective tool for detecting incipient outbreaks in populations of spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) in eastern North America (Ramaswamy *et al.*, 1983; Allen *et al.*, 1986; Sanders, 1988). In this system, male moths captured in sex pheromone-baited traps were used to predict relative densities of immature stages. Over a 21-year period in northwestern Ontario, catches were strongly correlated with late instar larval population densities in each subsequent year (Sanders 1988). Sex pheromone-based monitoring is in operational use for *C. fumiferana* in eastern North America. Other insect species for which adult male trap catch is related to immature density estimates include western hemlock looper, *Lambdina fiscellaria lugubrosa* (Hulst) (Lepidoptera: Geometridae) (Evenden *et al.*, 1995); gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Granett, 1974; Thorpe *et al.*, 1993); pine processionary moth, *Thaumetopoea pityocampa* Denis & Schiffermüller (Lepidoptera: Thaumetopoeidae) (Jactel *et al.*, 2006); Douglas-fir tussock moth, *Orygia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae) (Shepherd *et al.*, 1985); western spruce budworm, *Chorsistoneura occidentalis* Freeman (Lepidoptera: Tortricidae) (Sweeney *et al.*, 1990); potato tuberworm, *Phthorinaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (Shelton & Wyman, 1979); tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) (Tingle & Mitchell, 1981); European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) (Ngollo *et al.* 2000); lingonberry fruitworm, *Grapholita libertina* Heinrich (Lepidoptera: Tortricidae) (Hillier *et al.*, 2004); Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) (Lepidoptera: Tortricidae) (Asaro & Berisford, 2001) and corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Latheef *et*

al., 1991). This study represents the first attempt to establish quantitative relationships adults and immatures for *M. disstria* and *C. conflictana*

Malacosoma disstria

M. disstria is a widely distributed native defoliator in North America occurring from the Atlantic to Pacific coasts, and from Texas to northern Alberta (Stehr & Cook, 1968). *M. disstria* is a true generalist across its distribution but regional host specialization occurs (Parry & Goyer, 2004). Trembling aspen is the primary host tree in western Canada and across much of its northern range. *M. disstria* is a serious aspen defoliator and outbreaks occur in approximately ten-year cycles and can last from four to five years (Ives & Wong, 1988). *M. disstria* is univoltine and first instar larvae overwinter within the egg. Larvae emerge in the spring to coincide with aspen bud flush and feed upon the newly expanding foliage (Parry *et al.*, 1988). Pupation commences in June when larvae construct silk cocoons in folded leaves. Adults fly June to August in the boreal forest of western North America. Adult lifespan is ephemeral and mating occurs soon after eclosion. Mated female *M. disstria* oviposit their entire egg complement in one clutch on small twigs of the host (Fitzgerald, 1995).

Mating behaviour of *M. disstria* is not well documented. Female moths begin to eclose in late afternoon and call for mates almost immediately (Bieman & Witter, 1983). Male moths eclose earlier in the day and begin actively searching for females (Shepherd, 1979). Males are hypothesized to mate multiple times but strong evidence has not been shown to support this (Fitzgerald, 1995). Struble (1970) first demonstrated that female *M. disstria* attract males using sex pheromones. Chisholm *et al.* (1980) identified the major

component of the pheromone as (*Z*5, *E*7) dodecadienal (*Z*5*E*7-12Ald). It was later reported that a blend of *Z*5*E*7-12Ald, (*Z*5, *Z*7) dodecadienal (*Z*5*Z*7-12Ald) and *Z*7 dodecenal (*Z*7-12Ald) was more attractive to male moths than *Z*5*E*7-12Ald alone (unpublished data cited in Chisholm *et al.* 1982). A pheromone signal that contains 12-carbon chain compounds unsaturated at the 5 and 7-positions is common to at least eight species of the Lasiocampidae family (Ando *et al.* 2004). Schmidt et al. (2003) determined an attractive blend to monitor *M. disstria* populations in western Canada was a 100:1:10 ratio of *Z*5*E*7-12Ald, *Z*5*Z*7-12Ald and *Z*7-12Ald.

M. disstria is vulnerable at all life stages to a large number of natural enemies, which has important implications for population dynamics (Fitzgerald, 1995). Over 40 species of parasitoids and predators are associated with the egg, larval and pupal stages of *M. disstria* in the Canadian prairie provinces (Williams *et al.*, 1996). *M. disstria* is also susceptible to pathogenic infections by nuclear polyhedrosis virus and microsporidia. The microsporidian species *Nosema disstriae* (Thomson), *Pleistophora schubergi* Zwölfer and *Vairimorpha necatrix* (Kramer) (Protozoa: Microsporidia) have all been isolated from *M. disstria* populations (Wilson, 1984). *Nosema disstriae* is the most common species associated with *M. disstria* and infection causes increased mortality and reduced vigor in the laboratory (Wilson, 1977, 1979). However, the effect of microsporidia on natural populations of *M. disstria* is largely unknown.

Choristoneura conflictana

C. conflictana is also a native aspen defoliator distributed coast to coast in Canada and as far north as the southern Northwest Territories (Cerezke, 1992). Secondary hosts of *C. conflictana* include balsam poplar (*Populus balsamifera* L.), white birch (*Betula papyrifera* Marsh.), and willow (*Salix* spp.) (Prentice, 1955). Short-lived outbreaks occur periodically in aspen forests, and last from two to three years (Cerezke, 1992). *C. conflictana* is univoltine and overwinters as second instar larvae. Larvae emerge from hibernacula at the base of trees one to two weeks before aspen bud flush and ascend to the canopy to mine buds. After leaf expansion, larvae tie leaves together with silk and feed within the leaf structures for the rest of larval development. *C. conflictana* pupate within the leaf-structures and adults eclose approximately ten days later. Flight occurs in June and July in the boreal forest of western North America. Females oviposit at least one egg mass, generally on the upper surface of leaves, and larvae hatch after one to two weeks. First instar larvae feed gregariously within tied leaves before descending to the base of the tree where they moult to the second instar before overwintering (Prentice, 1955).

Mating biology of *C. conflictana* is largely unknown. Wickman (1963) reported that *C. conflictana* in California are diurnal and mate in the afternoon. The adult stage is estimated to last 10 days (Evenden, unpublished data), significantly longer than *M. disstria*. Weatherston *et al.* (1976) identified *Z*11-tetradecenyl aldehyde (*Z*11-14Ald) as an effective attractant for *C. conflictana* males and Weatherston *et al.* (1978) further demonstrated that *Z*11-14Ald alone attracted males. The pheromone of *C. conflictana* has

been identified from female effluvia as Z11-tetradecenal (Z11-14Ald) (Evenden & Gries, 2006) and is attractive throughout its geographic range (Weatherston *et al.*, 1978; Evenden & Gries, 2006). Z11-tetradecen-1-ol (Z11-14OH) was recovered as a minor component in female *C. conflictana* effluvia but did not affect male response when tested with Z11-14Ald (Evenden & Gries, 2006). A 14-carbon chain unsaturated at the 11-position is the typical molecular structure of sex pheromone for Tortricinae species (Ando *et al.*, 2004) and common to North American species of the *Choristoneura* genus (Silk & Kuenen, 1988).

C. conflictana is also attacked by a complement of natural enemies. Prentice (1955) listed over 30 dipteran and hymenopteran parasitoids reared from *C. conflictana*. At least eight microorganisms have been identified from field-collected *C. conflictana*, including viruses, fungi and microsporidia (Burke, 1982). Although three species of microsporidia are thought to have been observed in field-collected *C. conflictana* (Burke, 1982), *Nosema thomsoni* Wilson & Burke is the best described (Wilson & Burke, 1971). In addition, a microsporidium species was isolated in a laboratory colony of *C. conflictana* that has not previously been found in field populations (van Frankenhuyzen *et al.*, 2004). Infected female *C. conflictana* from natural populations are known to experience decreased longevity (Evenden *et al.*, 2006). Little more is known about sublethal microsporidian infections on *C. conflictana* but infection in other forest *Choristoneura* species decreased body size, longevity and fecundity, and can cause mortality (Thomson, 1958; Bauer & Nordin, 1989).

Thesis objectives and overview

The purpose of this thesis is to develop a combined sex pheromone-based monitoring system for two distantly related, native lepidopterous defoliators of aspen. Pheromone-based monitoring is a species-specific monitoring tool, and a combination of pheromone components for more than one species in a single lure represents a novel approach. *M. disstria* and *C. conflictana* are both major defoliators of trembling aspen in Alberta. In Chapter 2, I develop a tool that can detect changes in population density of both species simultaneously. I relate male moth capture with densities of immature stages of each species to develop a predictive model that can detect incipient outbreaks of *M. disstria* and *C. conflictana* in aspen stands in Alberta. The combined pheromone-based monitoring system has application for both forest managers and forest researchers. Forest managers will have a predictive monitoring tool that will increase efficiency of monitoring programs. Forest researchers will benefit from the development of a technique to study interspecific interactions and population dynamics of these ecologically important defoliators.

Pheromone-based monitoring systems have potential as an application tool to collect specimens for ecological studies. In Chapter 3, I undertake a complementary study to assess the condition of male *M. disstria* and *C. conflictana* captured in traps baited with the combined lure. I use measures of right forewing area and level of infection by microsporidian parasites as indicators of moth condition. I examine the effect of flight period and population density on male wing area and level of pathogen infection. Furthermore, I established a flight phenology for both species. Utilizing moths captured

in pheromone traps for ecological studies is not a common practice. I intend to show that pheromone traps have utility beyond enumeration of individuals.

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Chapter 2

Development of a combined sex pheromone-based monitoring system for *Malacosoma disstria* and *Choristoneura conflictana*

INTRODUCTION

Pheromone-based monitoring of insect populations requires that the number of adult insects captured correlate with local population density (Sanders, 1988). Such correlations have been observed for insect pests in many systems (Chapter 1, page 8). Species specificity of sex pheromone-baited traps implies that multiple trapping systems are required to monitor more than one pest in a given stand or crop. A sex pheromone-based monitoring tool that targets more than one pest species simultaneously and accurately predicts population densities of both species would be of practical and economic benefit. Such a system would require that pests have overlapping phenologies (i.e. flight season) and that targeted species are not repelled by heterospecific pheromone components. A single lure containing aggregation pheromones for several Coleoptera species has been successful for detection of forest (Scolytinae) (Miller *et al.*, 2005), orchard (Nitidulidae) (James *et al.*, 2000) and stored product pests (Curculionidae) (Wakefield *et al.*, 2005). To my knowledge, there has not been a sex pheromone-based monitoring system reported that uses a single blend, lure and trap to predict population density for more than one pest species simultaneously.

In this chapter, I present results for the development of a combined sex pheromone lure to monitor population densities of *Malacosoma disstria* Hübner and *Choristoneura conflictana* (Walker). Both species are important defoliators of trembling aspen *Populus tremuloides* Michx. Aspen is one of the most widely distributed tree species in North America (Farrar, 1995) and is of great economic and ecological importance in the western North American boreal forest (Hogg *et al.*, 2002). Insect defoliation of aspen,

especially by *M. disstria*, is known to reduce growth and cause tree mortality when combined with additional stressors such as drought (Candau *et al.*, 2002). Similar life history and ecology of these two species facilitates the development of a combined approach to population monitoring.

Distinct pheromone components for sexual communication in *M. disstria* and *C. conflictana* is evidence that unique chemical communication channels have evolved for each species and allow for the development of a combined approach to pheromone-based monitoring. The pheromone of *C. conflictana* is a 14-carbon chain unsaturated at the 11-position (*Z*11-14Ald) (Evenden & Gries, 2006). In contrast, *M. disstria* in western Canada is attracted to a blend of three compounds with 12-carbon chains (*Z5E*7-12Ald, *Z5Z*7-12Ald and *Z*7-12Ald) (Schmidt *et al.* 2003). The aim of this research is to use a single lure containing a synthetic pheromone blend comprised of both species' pheromone in a single trap to target two distantly related sympatric pest species. Preliminary work demonstrated that combining both species' pheromones into one lure is as attractive to males of each species as their respective pheromone alone (Evenden, 2005). My objective is to develop a predictive model to detect incipient outbreaks of both species in aspen stands in the Canadian boreal forest. Furthermore, the combined approach could provide a tool to study the individual population dynamics and the interactions between these two sympatric species. I tested the efficacy of the combined lure at varying densities of each species, the attractiveness of the combined lure to both species over the flight period, and compared the number of adult males captured in traps to estimates of abundance for immature stages.

METHODS AND MATERIALS

Study sites

In 2005, I established 23 field sites in three areas with differing population density for *M. disstria* and *C. conflictana* in central Alberta, Canada (Fig. 2.1). Sites with a high population density of one species served as low-density sites for the other with additional low-density sites for both species. Ten sites were located north of Wabasca, AB (56°18′N, 113°45′W) where an outbreak of *M. disstria* was in its fourth year (M. Maximchuk, pers. comm.). Seven sites were located within the infestation (Wabasca-high) and three were adjacent to the infestation. Five sites with low densities of both species were established approximately 70 km south of the *M. disstria* infestation (55°37′N, 113°26′W) (Wabasca-low). Ten sites with high-density *C. conflictana* populations (M.L. Evenden, pers. comm.) were established between Drayton Valley (53°13′N, 114°59′W) and Rocky Mountain House, Alberta (52°22′N, 114°55′W) (Rocky-Drayton). In 2006, I established five additional sites approximately 150 km east of the Wabasca infestation near Fort McMurray, AB (56°17′N, 111°39′W) to target a moderate population of *M. disstria* (Fort McMurray; Fig. 2.1). All study sites were at least 1 km apart and consisted of stands dominated by trembling aspen. Detailed site descriptions are located in Appendix A. Aspen stands with greater than 70% defoliation of the canopy were considered severely defoliated. Levels of defoliation at each site were recorded as none or severe (70-100% defoliation).

Pheromones

Pheromone blends were obtained from Phero Tech Inc. (Delta, BC) dissolved in HPLC grade hexane (Fisher Scientific Canada, Nepean, ON). I loaded pheromone blends into pre-extracted grey rubber septa (PheroTech Inc., Delta, BC) in the laboratory to produce four treatments in 2005 and one treatment in 2006 (Table 2.1). The amount of the *Z5Z7*-12Ald isomer in the *M. disstria* blend cannot be controlled during synthesis thus blends contained 0.65 and 3.1% *Z5Z7*-12Ald in 2005 and 2006 respectively (Table 2.1). Blends containing 1 – 10% of this component are known to attract male *M. disstria* (Palaniswamy *et al.*, 1983; Schmidt *et al.*, 2003; Evenden, 2005). Lures were stored in sealed jars at -20°C and transported to field sites in refrigerated containers.

Efficacy of combined pheromone lure

In 2005, I compared the attractiveness of lures containing both species' pheromones to lures containing each pheromone separately, and to a solvent control (Table 2.1). Each of the 23 sites served as blocks in a randomized block design. Treatment order was randomized within each site. Green, non-saturating Unitraps (Phero Tech Inc., Delta, BC) were baited at each site with lures containing one of four pheromone treatments (Table 2.1). Traps were hung at head height (~1.5 m), 50 m apart along a transect at the forest edge. A strip of Hercon® Vaportape II (Phero Tech Inc., Delta, BC) was used as a killing agent in each trap. Traps were deployed in early June and remained at sites until the end of August 2005 to span the flight season for both species. Catches were collected at two-week intervals and counted in the laboratory. Counts were totaled for each site to obtain a season-long catch for analyses. *M. disstria* trap catch was analyzed separately for high (n

= 6) and low (n = 17) density sites due to a marked difference in numbers of males captured among treatments between regions. Because the high-density population of *C. conflictana* collapsed in 2005, and trap catch was similar among areas, all sites were pooled for analysis (n = 22). I employed generalized linear models with Poisson errors to test if male moth capture differed among treatments (Crawley, 2002). Pheromone treatment and site were included as factors in the model. Individual pheromone treatments were compared with treatment contrasts using S-PLUS 7.0 (Insightful Corp.).

Longevity of combined pheromone lure

In 2005, I performed two lure longevity experiments during peak flight season for each species to determine attractiveness of the combined lure (Table 2.1) after exposure to field weathering for various time periods. I performed the experiments once in late June for *C. conflictana* in the Rocky-Drayton region, and once in mid-July for *M. disstria* in the Wabasca regions. Lures were loaded in the laboratory at weekly intervals and placed in a field enclosure on the University of Alberta campus for various periods prior to the commencement of each experiment. Lures were aged zero to five weeks for the experiment targeting *C. conflictana*, and one to nine weeks for the experiment targeting *M. disstria* to account for the protracted flight period of the latter species. Aged lures, and a hexane control, were randomly assigned to Unitraps and hung 50 m apart at head height (~1.5 m) along a transect at the edge of forest stands, and replicated at six sites for the experiment targeting *C. conflictana* and seven sites for the experiment targeting *M. disstria*. Traps remained in the field for two weeks and total catch was counted in the laboratory. A generalized linear model with Poisson errors was performed for each

experiment using S-PLUS 7.0 (Insightful Corp.) to detect an effect of lure age on lure attractiveness (Crawley, 2002). Lure age was treated as a continuous variable.

Immature sampling and predictive capabilities of the combined pheromone lure

M. disstria

M. disstria pupae and egg masses were sampled during the 2005 and 2006 field seasons to estimate abundance. Pupae were sampled at 23 and 25 sites in 2005 and 2006 respectively. Counts were made in late-June using a timed-search for cocoons (Roland & Taylor, 1997). A transect at the stand edge was walked for 20 minutes at each site and all cocoons within 2 m off the ground were counted. If 100 cocoons were counted in less than 20 minutes, the search was stopped and the number of cocoons that would have been counted in 20 minutes was extrapolated. *M. disstria* egg masses oviposited in 2005 and 2006 were sampled at 15 and 14 sites, respectively. Three co-dominant aspen trees were felled at each site in mid-October of each year and one haphazardly selected branch was removed from each of the lower, middle and upper portion of the tree crown. Egg masses were collected from the terminal 0.75 m of each branch and transported to the laboratory for counting. Total number of eggs per site was used for analysis. The combination of egg and pupal counts permitted three generations of *M. disstria* to be sampled through 2005 and 2006.

C. conflictana

C. conflictana late instar larvae and egg masses were sampled with a similar tree-felling protocol to that used to sample *M. disstria* egg masses. Larvae were sampled in late May

of 2005 at 15 sites. No larvae were found at sites in the Wabasca regions in 2005, consequently sampling was limited to eight sites in the Rocky-Drayton region in 2006. Egg masses were sampled at 19 sites in mid-July 2005 but were rarely found, resulting in numbers too low for meaningful statistical analysis. Egg masses were not sampled in 2006 and therefore only two generations of *C. conflictana* were sampled through 2005 and 2006.

Predictive model

All possible combinations of male trap capture and immature counts for each species were regressed to test for significant relationships within and between generations. Data were tested for linear effects using generalized linear model regressions with Poisson errors (S-PLUS 7.0, Insightful Corp.). Additional non-linear effects were assessed by inserting a squared term into the model and comparing the linear and non-linear models using ANOVA. Estimate of fit for models was determined by calculating the proportion of deviance explained by the model (Crawley, 2002). *M. disstria* pupal counts were log transformed to provide better resolution of the data in which counts spanned several orders of magnitude.

RESULTS

Efficacy of combined pheromone lure

Traps baited with the combined lure containing both species' pheromone (Table 2.1) captured male *M. disstria* at both high- and low-densities. There was a strong effect of pheromone treatment on the number of males captured at low-density sites (Fig. 2.2A).

There was no difference in male capture between traps baited with *M. disstria* pheromone alone and the combined lure (Fig. 2.2A). No *M. disstria* males were captured in the *C. conflictana* pheromone- or control-baited traps at low-density sites. At high-density sites equal numbers of *M. disstria* males were captured in all traps (Fig. 2.2B). Trap catch was lower than expected for high-density sites and there was no difference in catch between high- and low-density sites for traps baited with the combined lure ($F_{1,21} = 0.58$, $P = 0.31$).

Pheromone treatment had a strong effect on the number of *C. conflictana* males captured in traps at all sites (Fig. 2.3). An equal number of males were captured in traps baited with the combined lure and those baited with *C. conflictana* lures (Fig. 2.3). Traps baited with *M. disstria* pheromone and solvent control lures did not capture any *C. conflictana* males.

Lure longevity of combined pheromone lure

There was a negative effect of lure age (0 – 9 weeks) on the capture of *M. disstria* males in traps baited with the combined lure (Fig. 2.4A). There was no effect of lure age (0 – 5 weeks) on the capture of male *C. conflictana* (Fig. 2.4B).

Immature sampling and predictive capabilities of the combined pheromone lure

M. disstria

Pupal counts were not related to numbers of captured males within the same generation in 2005 (Fig. 2.5A). Sites defoliated in 2005 and 2006 had high pupal counts and the

number of males captured in pheromone-baited traps was no different from those sites with no visible defoliation (Fig. 2.5A). In 2006, pupal counts were significantly related to male trap capture in the same generation through a non-linear relationship (Fig. 2.5B). Defoliation occurred at more sites in 2006 than in 2005, and again all defoliated sites had high pupal counts but relatively low male trap capture (Fig. 2.5B). Numbers of eggs sampled in the fall of 2005 were inversely linearly related to males within the same generation captured the following summer (Fig. 2.5C). Egg counts in 2005 were highest at defoliated sites (Fig. 2.5C).

Pupal counts in 2005 were related to numbers of captured males in the subsequent generation in 2006 through a non-linear relationship (Fig. 2.6A). Sites defoliated in 2005 and 2006 had high pupal counts and low trap catch while sites defoliated only in 2006 had lower pupal counts and moderate to high trap catch (Fig. 2.6A). Numbers of captured males in 2005 predicted numbers of eggs in the next generation oviposited later in the year through a non-linear relationship (Fig. 2.6B). Sites defoliated in 2005 and 2006 mostly had high egg counts and moderate trap catch (Fig. 2.6B). Adult captures in 2005 did not predict pupal counts in 2006 (Fig. 2.6C), nor did adult captures in 2006 predict numbers of eggs in the subsequent generation in 2006 (Fig. 2.6D). In both cases, defoliated sites had high pupal or egg counts, but no trend was evident between defoliation and male captures (Fig. 2.6C, D).

C. conflictana

Larval counts in the summer of 2005 were not related to numbers of males captured later that summer (Fig. 2.7A). However, there was a strong, linear relationship between larval counts in 2006 and male moths in the same generation (Fig. 2.7B). Larval counts in the summer of 2005 were related to numbers of captured males in 2006 through a linear relationship (Fig. 2.8A). Numbers of males captured in 2005 did not predict larval counts in 2006 (Fig. 2.8B).

DISCUSSION

A sex pheromone-based monitoring system that targets two forest insect pests simultaneously using one pheromone blend, one lure and one trap is a novel approach with economic potential and ecological application. This study demonstrated the feasibility of a combined pheromone-based approach to simultaneously monitor populations of *M. disstria* and *C. conflictana*. The combined lure was as attractive to both *M. disstria* and *C. conflictana* males as traps baited with each species' pheromone alone. My findings confirm that the lures release the correct amount and component ratio of each species' pheromone, and there are no antagonistic effects of heterospecific pheromone components to the males' response at the rates tested (Evenden, 2005).

At high-density *M. disstria* sites, all traps captured *M. disstria* males, including the solvent-baited control traps. This was likely due to the extremely high number of adults in flight and the high probability that they would encounter a trap. Pheromone contamination among traps was ruled out as a potential factor because the lures and traps

were handled in the same manner at all sites, and male *M. disstria* were only captured in control traps at high-density sites. *M. disstria* trap catch was low at all sites in 2005 compared to 2006 regardless of population density. Previous studies in which *M. disstria* lures contained the three-component blend with 1% *Z5Z7*-12Ald (Schmidt *et al.*, 2003) showed findings similar to my 2005 results. Evenden (2005) found *M. disstria* captures in traps baited with the same combined lure, but containing 4% *Z5Z7*-12Ald, was comparable to the 2006 trap catch in the current study in which lures contained 3.1% of this component. Although the amount of this isomer cannot be controlled in the synthesis of the pheromone, further development of the combined system should control for *M. disstria* pheromone blends between years. Furthermore, (*Z5*, *E7*) dodecadien-l-ol (*Z5E7*-12OH) has been identified as a minor pheromone component from female gland extracts (Chisholm *et al.*, 1980) but is not included in the current commercially available monitoring blend for *M. disstria* (Schmidt *et al.*, 2003). This component has not yet been tested with the tertiary blend but addition of *Z5E7*-12OH to the combined blend should be tested in future studies to improve attractiveness of the combined lure to *M. disstria*. Capture of male *C. conflictana* in traps baited with the combined lure also varied greatly between years. Trap catch from 2006 was higher than 2005 in this study and was comparable to previous results using the combined lure (Evenden, 2005). Direct comparison of captures between years is not possible due to natural variation in population density and climatic factors affecting pheromone response and moth flight. Variation in trap catch between years should not hinder efficacy of the combined monitoring tool for either species because the goal of a pheromone-based monitoring

system is to relate numbers of adult captures to population density estimates and not to maximize absolute trap catch (Jactel *et al.*, 2006).

For season-long monitoring, combined lures need to be attractive throughout the flight period. In the present study, the combined rubber septa lure was less attractive to *M. disstria* males after being field-aged for approximately five weeks. Lure age had no effect on capture of *C. conflictana* males when aged up to five weeks. Pheromone release rates from rubber septa decrease exponentially with time (Butler & McDonough, 1981) and are affected by temperature and wind speed (McDonough, 1991). Thus, older lures may be less attractive to male *M. disstria* due to a low release rate of remaining pheromone. Changes in component composition over time due to differential loss, may also decrease lure attractiveness (Môttus *et al.*, 1997). This is particularly problematic for aldehydes (McDonough, 1991) such as the pheromone components of these species. A 14-carbon monounsaturated aldehyde has a half-life of approximately 43 days on rubber septa (Heath *et al.*, 1986). Accordingly, there should be sufficient *Z*11-14Ald remaining on the septa to attract *C. conflictana* males to lures aged for five weeks. The major component in the *M. disstria* blend is a 12-carbon diunsaturated aldehyde. Decreasing carbon chain length and increasing unsaturation both reduce the half-life of aldehydes (Heath *et al.*, 1986). As a result, rubber septa lures loaded with *Z5E*7-12Ald will have a half-life much less than 43 days. This estimation matches the observed decrease in attractiveness of the combined lure to male *M. disstria* (~35 days). However, lures should remain attractive to *M. disstria* for the duration of the flight period given that Schmidt *et al.* (2003) showed little difference in male captures in traps baited with lures containing either 67 or 390 µg

of the tertiary blend. In 2005 and 2006, both the combined and *M. disstria* lures loaded with 100 µg of the main component continued to attract males until the traps were removed at the end of August. Future work should test the effect of age of the combined lure with higher loadings of *M. disstria* pheromone.

C. conflictana larval counts were significantly related to numbers of males captured in traps both within and between generations. Although larval samples were strongly related to adult trap catch, significant relationships were driven by one site. Different population levels were targeted for monitoring and sampling; however, the high-density *C. conflictana* population declined at the onset of the study and reduced the breadth of population densities sampled. *C. conflictana* outbreaks are short-lived (Cerezke, 1992). It is therefore difficult to identify areas with incipient outbreaks with which to increase the range of population densities for study. A three-tree sample per site, combined with the narrow range of population densities sampled, may account for the lack of significant relationships between male captures and larval counts in the subsequent generation.

M. disstria egg and pupal counts were related to numbers of captured males both within and between generations. In addition, high pupal counts were indicative of severe defoliation. Because *M. disstria* pupal counts are related to numbers of male captures, the potential exists for the combined lure to predict changes in defoliation; however, this remains to be directly tested. These data also show that pupal counts could predict incipient outbreaks of *M. disstria.*

The relationships between numbers of adult male captures and immature counts of *M. disstria* are either linear or curvilinear. Pheromone trap captures of *M. disstria* level off at high population densities when density is estimated by pupal counts (Roland, 2005) and by visual defoliation (Schmidt *et al.*, 2003). My results show that *M. disstria* trap catch not only levels off at population high densities but also declines at extremely high density. Low capture of *M. disstria* males in pheromone-baited traps at sites with high population densities may indicate that a high level of naturally produced ambient pheromone disrupts males from locating a synthetic pheromone source. *M. disstria* mate finding was disrupted by permeating the air with synthetic pheromone (Palaniswamy *et al.*, 1983). This phenomenon, referred to as the "competition effect" (Cardé, 1979), has been demonstrated experimentally in codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Howell, 1974) and the maize stemborer *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) (Unnithan & Saxena, 1991). In addition, *M. disstria* males may rely on visual cues to locate females (Palaniswamy *et al.*, 1983) and therefore may not be attracted to a trap when calling females are available. Reduced capture of *M. disstria* males at high-density sites may also be explained by high parasitism rates of larvae (Fitzgerald, 1995) resulting in pupal and egg samples that overestimate adult abundance. Alternatively, the synthetic pheromone may be less attractive to male *M. disstria* than a natural plume emitted by calling females. Although the combined lure does not attract male *M. disstria* in proportion to the actual population density at outbreak levels, the main objective of the model is to predict changes in population density at low levels and detect incipient outbreaks.

Several factors may account for the unexpected, weak, or lack of relationships between *M. disstria* trap capture and immature samples. There is large variation in numbers of egg masses and numbers of eggs per mass both within and among forest stands for *M. disstria* (Hodson, 1941; Witter *et al.*, 1975). This variation was observed at both high and low density sites in this study. It was expected that egg counts would have the same curvilinear relationship with male trap capture as pupae. However, the difference in the resulting curves may be explained by variation in numbers of eggs and masses and large variation in male trap catch among sites even when sites were partitioned by level of defoliation. Variation in stand composition among sites may also weaken relationships (Allen *et al.*, 1986; Sanders, 1988; Sweeney *et al.*, 1990). Although all sites in this study were aspen-dominated, each site had slightly different tree species composition and age (Appendix 1). It is possible that sites with a greater number of large aspen trees could yield more insects due to greater foliage biomass (Sanders, 1988; Sweeney *et al.*, 1990). Pheromone traps attract adult males at a stand level; therefore, an increased number of immature samples in conjunction with greater control of site composition heterogeneity could increase the strength of relationships between adults and immature stages. Mortality from predation and parasitism, and adverse climatic conditions may also reduce the strength of relationships between adult catch and samples of immature stages. The effect may be particularly acute between generations that have longer inter-sample periods (Jactel *et al.*, 2006). *M. disstria* infestations are known to have high rates of parasitism (Fitzgerald, 1995). Inclusion of a weighting factor to account for parasitism rate of each life stage in the model, may improve its predictive capabilities (Evenden *et al.*, 1995).

The goal of this study was to develop the combined lure so that numbers of captured males of both species could predict immature stage abundance in the subsequent generation and detect changes in population density. *C. conflictana* larval counts were related to male captures but trap capture did not predict larval densities in the subsequent generation. Numbers of captured *M. disstria* males predicted numbers of eggs in the subsequent generation but not in the relationship expected. *M. disstria* pupae provided the best relationships with male captures through a curvilinear relationship. A technique based on pupal counts may be better able to detect changes in population density of *M. disstria* but would be more time consuming, less cost effective and potentially less sensitive at low densities than pheromone trapping. With the improvements discussed above, the combined system could be further developed to successfully predict incipient outbreaks of both species in the boreal forest of western Canada. Climate warming will likely exacerbate the occurrence of insect outbreaks in the boreal forest (Logan *et al.*, 2003) resulting in *M. disstria* and *C. conflictana* potentially driving aspen stand dynamics (Volney & Hirsch, 2005). Therefore, the combined monitoring system presents a timely and cost effective approach for forest managers and researchers.

Table 2.1. Sex pheromone blends and quantities used in treatments for monitoring adult male *M. disstria* and *C. conflictana*.

Experiment (year)	Treatment	Pheromone blend	Quantity (µg) In lure
Efficacy of combined lure (2005)	*M. disstria*	*Z5E7*-12Ald	100
		Z5Z7-12Ald	0.65
		Z7-12Ald	10
	C. conflictana	Z11-14Ald	100
	Combined	*Z5E7*-12Ald	100
		Z5Z7-12Ald	0.65
		Z7-12Ald	10
		Z11-14Ald	100
	Control	Hexane	100
Longevity of combined lure (2005)	Combined	*Z5E7*-12Ald	100
		Z5Z7-12Ald	0.65
		Z7-12Ald	10
		Z11-14Ald	100
	Control	Hexane	100
Predictive capabilities of combined lure (2005)	Combined	*Z5E7*-12Ald	100
		Z5Z7-12Ald	0.65
		Z7-12Ald	10
		Z11-14Ald	100
Predictive capabilities of combined lure (2006)	Combined	*Z5E7*-12Ald	100
		Z5Z7-12Ald	3.1
		Z7-12Ald	10
		Z11-14Ald	100

Figure 2.1. Map of regions where study sites were located in Alberta, Canada.

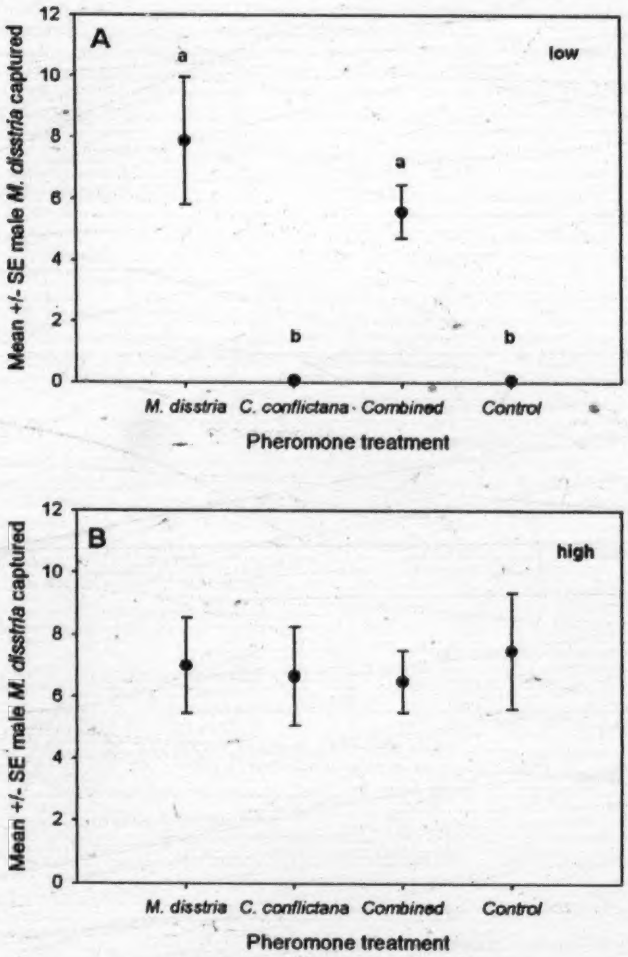

Figure 2.2. Effect of pheromone treatment on mean number of *M. disstria* males captured at (A) low-density and (B) high-density sites in traps. Male counts are based on season-long catch. Means for treatments with different letters above them are significantly different (GLM with Poisson errors; (A) $F_{3,64} = 29.95$, $P < 0.001$; (B) $F_{3,20} = 0.083$, $P = 0.97$; treatment contrasts $P < 0.05$).

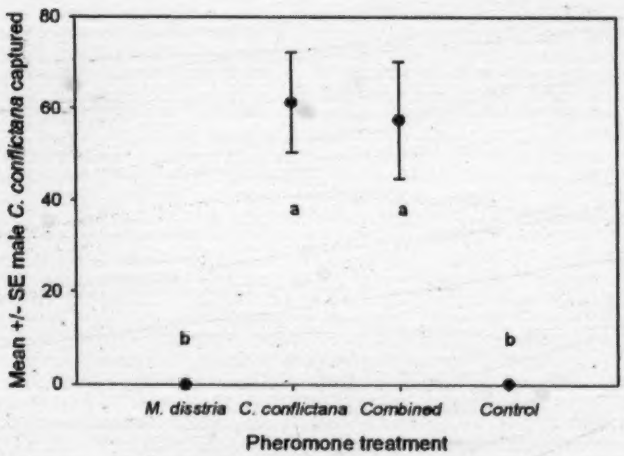

Figure 2.3. Effect of pheromone treatment on mean number of *C. conflictana* males captured in traps. Male counts are based on season-long catch. Means for treatments with different letters above them are significantly different. (GLM with Poisson errors: $F_{3,84} = 44.20$, $P < 0.001$; treatment contrasts, $P < 0.05$).

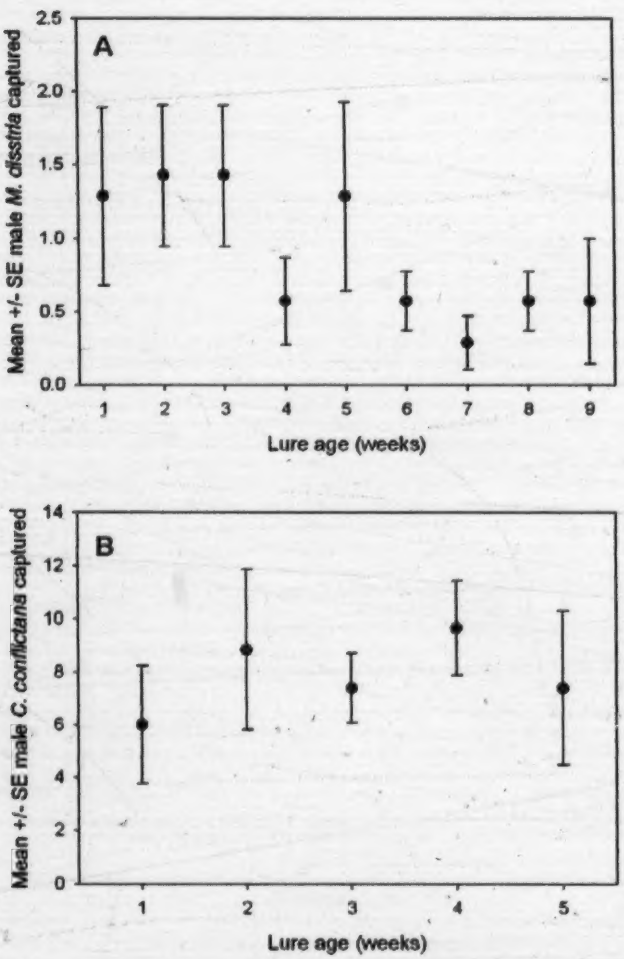

Figure 2.4. Effect of lure age on mean number of (A) *M. disstria* (GLM with Poisson errors, $F_{1,55} = 5.22$, $P = 0.027$) and (B) *C. conflictana* (GLM with Poisson errors, $F_{1,26} = 0.020$, $P = 0.89$) males captured in traps with field-aged lures containing the combined treatment.

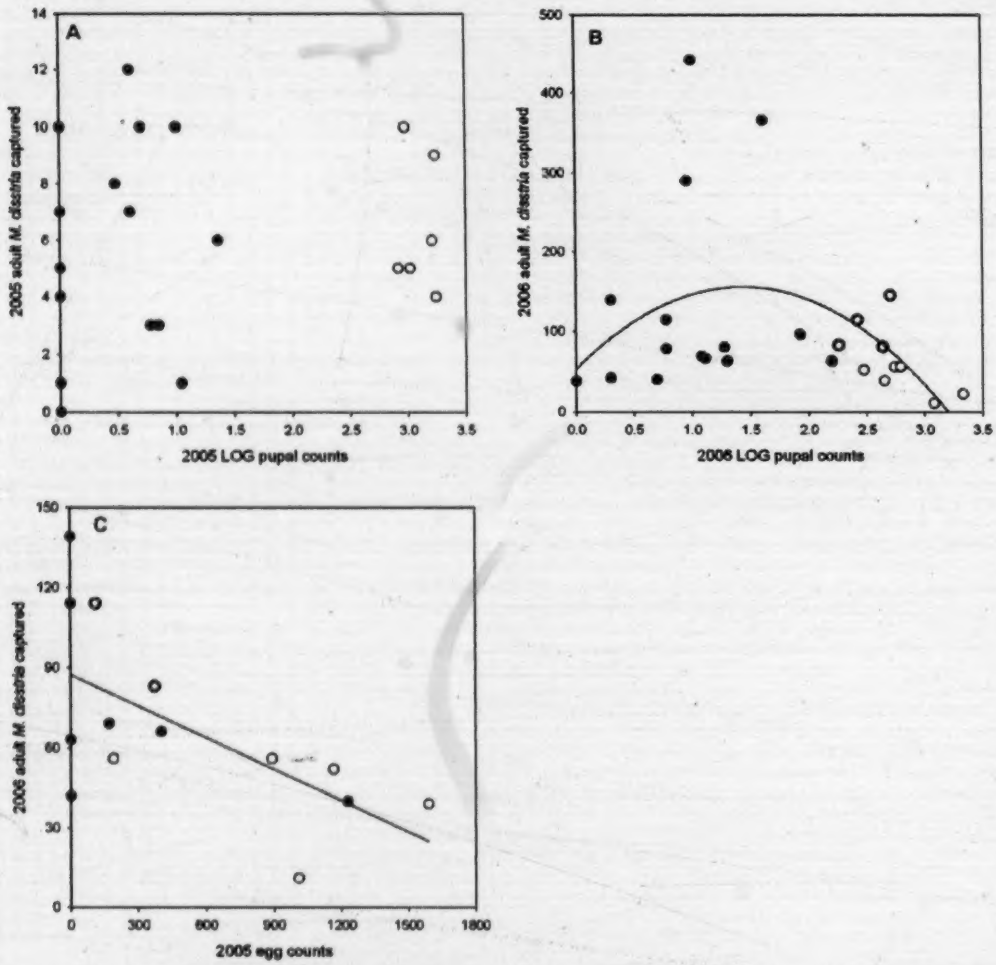

Figure 2.5. Relationships between immature stages of *M. disstria* and males from the same generation captured in traps baited with the combined pheromone lure. Closed circles indicate sites with no defoliation in 2005 or 2006; open circles indicate severely defoliated sites (i.e. > 70%) in 2005 and 2006; bold open circles indicate severe defoliation in 2006 only. (A) Relationship between pupal counts and male captures in 2005 (n = 23); y =1.68 + 0.065x, proportion of deviance explained = 0.01, $P = 0.62$. (B) Relationship between pupal counts and male captures in 2006 (n = 25); y = 3.89 + 1.77x - $0.62x^2$, proportion of deviance explained = 0.31, $P = 0.027$. (C) Relationship between numbers of total eggs sampled in 2005 and male captures in 2006 (n = 14); y = 4.50 - 6.8×10^{-4}x, proportion of deviance explained = 0.42, $P = 0.0087$.

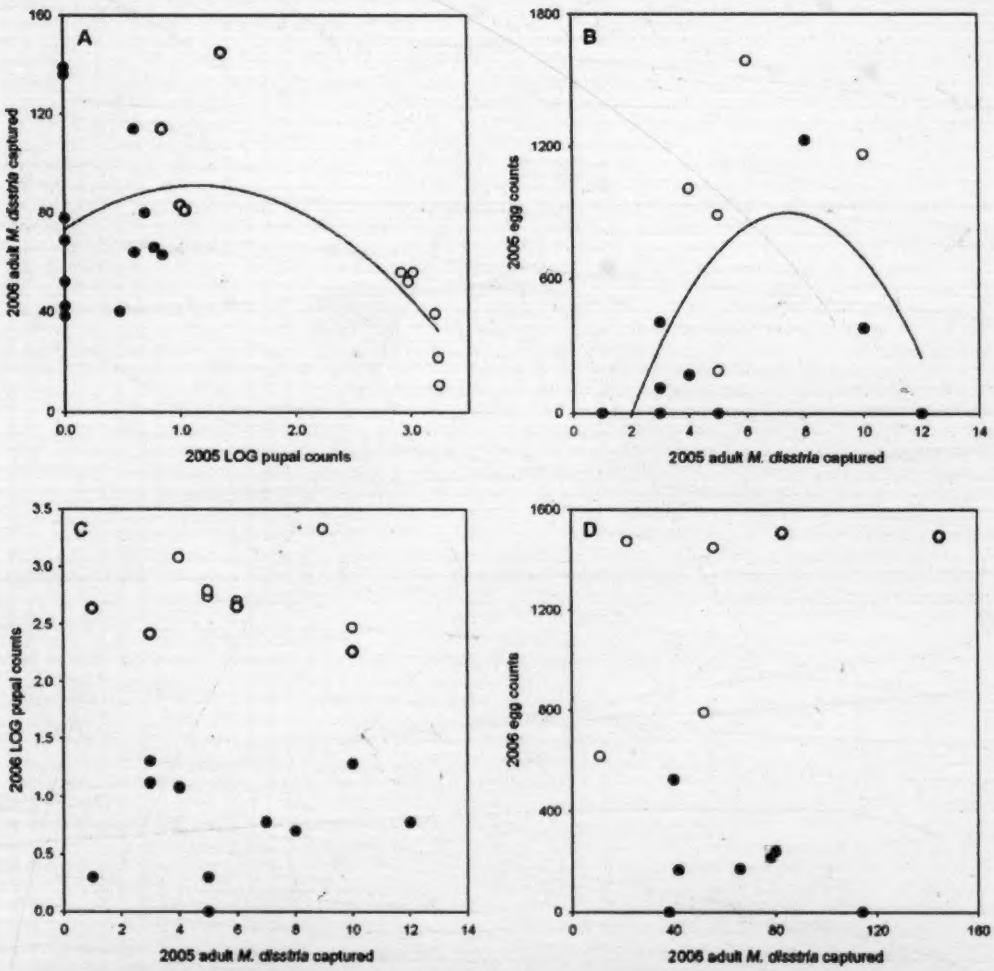

Figure 2.6. Relationships between generations for immature stage of *M. disstria* and males from traps baited with the combined pheromone lure. Closed circles indicate sites with no defoliation in 2005 or 2006; open circles indicate sites severely defoliated (i.e. > 70%) in 2005 and 2006; bold open circles indicate severe defoliation in 2006 only. (A) Relationship between pupal counts in 2005 and male captures in 2006 (n = 23); $y = 4.29 + 0.42x - 0.20x^2$, proportion of deviance explained = 0.36, $P = 0.014$. (B) Relationship between male captures in 2005 and numbers of total eggs sampled in 2005 (n =14); $y = 0.97 + 1.68x - 0.11x^2$, proportion of deviance explained = 0.49, $P = 0.019$. (C) Relationship between male captures in 2005 and pupal counts in 2006 (n = 20); $y = 0.50 + 0.0086x$, proportion of deviance explained = 0.001, $P = 0.85$. (D) Relationship between male captures in 2006 and numbers of total eggs sampled in 2006 (n = 13); $y = 6.34 + 0.0025x$, proportion of deviance explained = 0.01, $P = 0.73$.

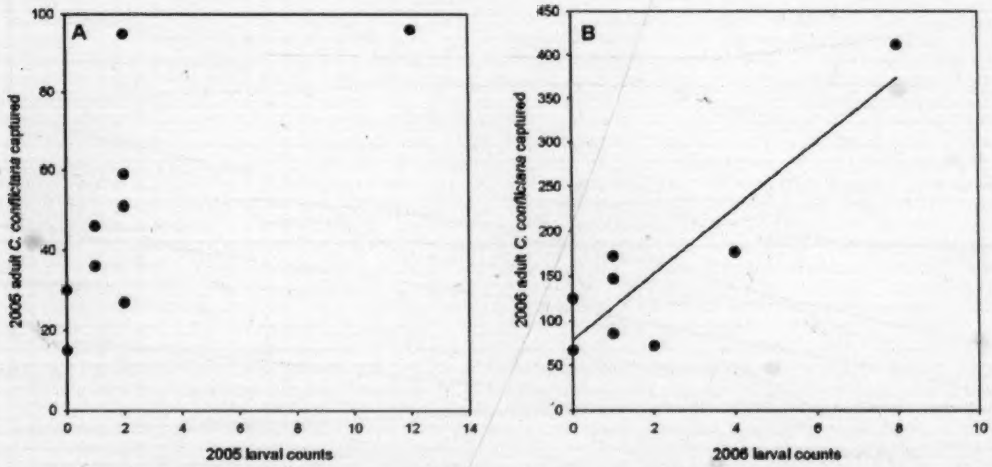

Figure 2.7. Relationships between larval counts of *C. conflictana* in early summer and males captured in the same generation later that summer in traps baited with the combined pheromone lure. (A) 2005 (n = 9); y = 3.68 + 0.080x, proportion of deviance explained = 0.40, $P = 0.076$. (B) 2006 (n = 8); y = 4.56 + 0.18x, proportion of deviance explained = 0.79, $P = 0.0032$.

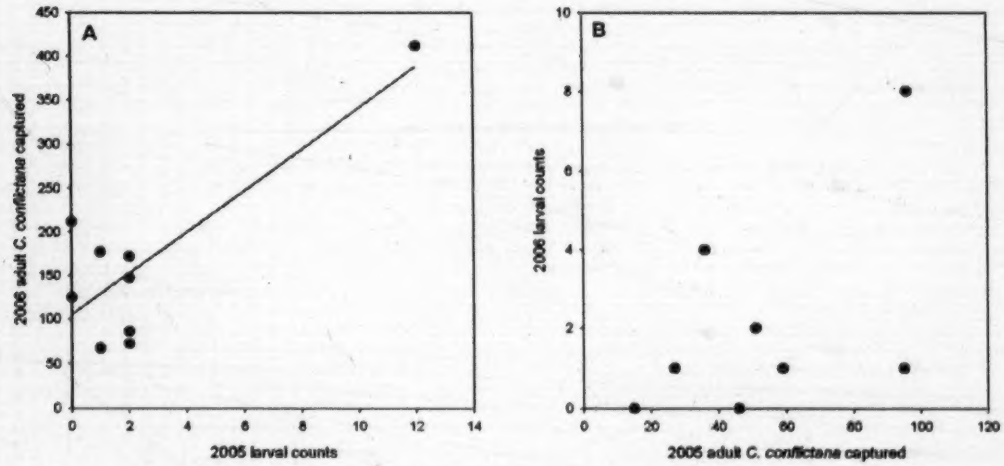

Figure 2.8. Relationships between larval counts of *C. conflictana* in early summer and males captured from the subsequent generation in the following summer in traps baited with the combined pheromone lure. (A) Relationship between larval counts in 2005 and male captures in 2006 (n = 9); y = 4.77 + 0.10x, proportion of deviance explained = 0.60, $P = 0.014$. (B) Relationship between male captures in 2005 and larval counts in 2006 (n = 9); y = 3.75 + 0.092x, proportion of deviance explained = 0.24, $P = 0.22$.

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Chapter 3

Use of pheromone traps to monitor moth condition

INTRODUCTION

Pheromone trapping is primarily a tool for use in IPM to support insect pest control strategies. Insects captured in pheromone-baited traps are counted to detect pest presence or absence, to determine a threshold catch, or to estimate population density (Howse *et al.*, 1998). However, captured insects also represent a potential source of specimens for ecological studies. For instance, the condition of captured adult insects may provide insight on the state of the local population. Insect size and health may indicate status of an infestation while simultaneously revealing information about environmental conditions and species interactions. Pheromone traps may be checked at intervals through the duration of a species' flight season to examine temporal effects on condition and establish flight phenology of pest species. This type of information has implications for population dynamics research in which moth condition may be predictive of phases in population cycles. In this chapter, I demonstrate some ecological applications of pheromone trapping with the combined sex pheromone-based monitoring tool discussed in Chapter 2. Adult male *Malacosoma disstria* Hübner and *Choristoneura conflictana* (Walker) captured with the monitoring system are examined more closely to measure moth quality of these two species that undergo cyclic changes in population density.

In this study, condition of *M. disstria* and *C. conflictana* males is assessed by wing area and level of infection by an intracellular parasite. Microsporidia (Protozoa: Microsporidia) are unicellular parasites that affect many forest Lepidoptera (Maddox *et al.*, 1998). Microsporidia occur in the environment as resistant spores until ingested by larvae, although most species can be transmitted horizontally or vertically via trans-

ovariole transmission from mother to offspring (Maddox *et al.*, 1998). Microsporidian species are found in natural populations of both *M. disstria* (Thomson, 1959) and *C. conflictana* (Wilson & Burke, 1971). Sublethal microsporidian infections of Lepidoptera are associated with deceased body size, fecundity and longevity (Thomson, 1958a; Gaugler & Brooks, 1975; Wilson, 1977a; Wilson, 1979; Bauer & Nordin, 1989; Evenden *et al.*, 2006). The level of microsporidian infection in *M. disstria* increases as population outbreaks progress and insect density increases (Fitzgerald, 1995). Thus incidence and level of infection potentially have implications for population dynamics of forest Lepidoptera.

Wing area of Lepidoptera is a useful measure of condition because adult size is associated with degree of fecundity (Honek, 1993; Evenden *et al.*, 2006) and wing size is related to flight ability (Hill *et al.*, 1999). Moth wing measurements have been used in several studies as an indicator of population quality (Bellinger *et al.*, 1990; Du Merle & Cornic, 1991; Hoffman *et al.*, 2002) as insect size generally decreases during population outbreaks (Elkinton & Liebhold, 1990; Klemola *et al.*, 2004). In addition, microsporidian infection level and wing area are not independent of each other (Bauer & Nordin, 1989). Pheromone trapping provides a way to examine moth condition and provides an opportunity to study density and temporal effects on the state of adult male Lepidoptera.

I performed a study to examine the effect of flight period and population density on the condition of *M. disstria* and *C. conflictana* male moths. Males were collected with the combined pheromone-based monitoring system developed in Chapter 2. I determined the

flight phenology of each species by emptying traps at regular intervals throughout the flight season to enumerate the catch. I hypothesized that both flight period and population density would affect moth condition. Specifically, I predicted that males captured at sites with high population densities would have smaller wings and higher levels of microsporidia infection than males captured at low-density sites. I also expected to see smaller males with a higher level of microsporidia infection later in the flight period (Eveleigh *et al.*, 2007). These factors are not independent of each other; thus I predicted an interaction between collection date and infection level on wing area. These data in conjunction with the flight phenology of *M. disstria* and *C. conflictana* males will provide ecological information on two important forest pests with a non-traditional application of pheromone trapping.

METHODS AND MATERIALS

Study sites

Field sites were established in 2005 and 2006 as part of a larger pheromone-based monitoring study for *M. disstria* and *C. conflictana* in central Alberta, Canada (see Chapter 2; Fig. 2.1). Geographic coordinates and detailed site descriptions are provided in Appendix A. To test the effect of population density on moth condition in each species, sites were established in two regions in 2005 with either a high-density population of *M. disstria* or *C. conflictana*. I established sites at a third region with endemic populations for both species. High-density sites for one species served as low-density sites for the other species. Seven sites targeting high-density populations of *M. disstria* were located north of Wabasca, AB (Wabasca-high) where an outbreak of *M.*

disstria was in its fourth year. Five sites with endemic populations of both species were established approximately 70 km south of the *M. disstria* infestation (Wabasca-low). Nine sites targeting high-density *C. conflictana* populations were established between Drayton Valley and Rocky Mountain House, AB (Rocky-Drayton). In 2006, five additional sites were established to target a moderate population of *M. disstria* approximately 150 km east of the Wabasca infestation near Fort McMurray, AB (Fort McMurray). All study sites were at least 1 km apart.

Pheromone trapping

Pheromone traps were deployed at each site before flight occurred for either species in 2005 and 2006. Details of the pheromone trapping protocol are described in Chapter 2 as part of the monitoring study. Unitraps (Phero Tech Inc., Delta, BC) were baited at each site with a combined pheromone lure that simultaneously targeted *M. disstria* and *C. conflictana* (Table 2.1). Pheromone blends were obtained from Phero Tech Inc. (Delta, BC). In 2005, traps were deployed on 7 June in the Rocky-Drayton region and on 8-9 June in the Wabasca regions. In 2006, traps were deployed on 7 June in the Rocky-Drayton region and on 8-9 June in the Wabasca-high, Wabasca–low, and Fort McMurray regions. All traps were taken down 29-31 August 2005 and 28-30 August 2006. To test the effect of flight period on the condition of each species, captured males were collected at approximately two-week intervals in 2005 and three-week intervals in 2006. Logistical constraints prevented more frequent collection. Captured males were emptied from traps into plastic bags and stored at -20°C until they were used for data collection.

Measuring moth condition

Male samples

I examined male *M. disstria* and *C. conflictana* captured at each site on each collection date to assess moth condition. Five moths per site at each collection date were sampled when possible for a cumulative sample of 30 males of each species per region and collection date in each year. Males were haphazardly selected from bags of frozen specimens. If a selected male had damaged wings it was discarded and another selected. The same specimen was used for wing area and microsporidia measurements. The high-density population of *C. conflictana* collapsed in 2005, thus density-dependent effects on condition could not be studied. Therefore only *C. conflictana* collected in the Rocky-Drayton region were assessed for both years. In 2005, there were insufficient numbers of male *M. disstria* captured at any site to take measurements of condition indices. *M. disstria* from all regions were examined in 2006. Only *M. disstria* moths captured at sites near Rocky Mountain House (55° 22′ N, 114° 59′ W) in the Rocky-Drayton region were measured so that all four regions had a similarly sized geographic area.

Wing area

To determine wing area for captured male *M. disstria* and *C. conflictana*, I removed the right forewing with forceps from randomly selected males. Wings were glued onto white, standard printer paper stapled to cardboard for support, using a glue stick (Power Glue Stick, Mungyo®). I noted site and date below each wing. To protect glued wings, sheets of paper were covered with acetate sheets. Sheets with wings were scanned with a HP

Scanjet 4070 Photosmart scanner. Wing images were converted to 8-bit grayscale and then binary to produce black wing images with sharper edges in ImageJ 1.34s (National Institute of Health, USA). Wings with small pieces missing were filled in using the 'Pencil' function in ImageJ to best match existing wing edges. Wings with large pieces missing were discarded. Wings were automatically measured using ImageJ to the nearest 0.001 cm^2 (ImageJ 1.34s, National Institute of Health).

Microsporidia infection level

To assess microsporidia infection level, I removed abdomens of male *M. disstria* and *C. conflictana* and placed them into 1 ml microcentrifuge tubes with 0.2 ml of distilled water. I ground the abdomens with a Teflon rod and homogenized the solution with a vortex before pipetting 25 µl onto a glass microscope slide. Slides were examined under 400x magnification for presence of microsporidia spores. Large spores (~2.0 x 4.5 µm) from *M. disstria* were found and presumably from the genus *Nosema* (Thomson, 1959). Microsporidian spores from *C. conflictana* were markedly smaller (~1.5 x 2.5 µm) and morphologically similar to *Cystosporogenes* spp. (van Frankenhuyzen *et al.*, 2004). Ten fields of view were examined for each slide and the level of infection for each male was scored as none, low (1-10 spores per field of view), or high (>10 spores per field of view).

Statistical analyses

Due to few collection dates throughout the flight period, date was treated as a categorical variable. I tested the effect of collection date and geographical region on wing area of

male *M. disstria*, and the effect of collection date and microsporidia infection level on wing area of male *C. conflictana* using a GLM (SPSS 11.0.3, SPSS Inc.) followed by a Tukey HSD test to for pairwise comparisons of region and date. A chi-square likelihood test was employed to test for independence between level of infection and collection date.

Flight phenology

I plotted the flight phenology of *M. disstria* and *C. conflictana* for 2005 and 2006. The number of males captured per site was calculated and plotted against collection date for each region in each year. Cumulative degree-days for each region were calculated and plotted against calendar date to determine if differences in phenology between regions were due to temperature. I used the single sine method (Zalom *et al.*, 1983) to calculate degree-days using the program 'ddsine' (Snyder, 2001). The lower developmental threshold temperature used in the program was 5°C for both *M. disstria* (Ives, 1973; Parry *et al.*, 1998; Roland *et al.*, 1998) and *C. conflictana* (Parry *et al.*, 1997). Degree days were accumulated from 1 January of each year. Maximum and minimum temperature data for each region were acquired from Environment Canada (http://www.climate. weatheroffice.ec.gc.ca/climateData/ canada_e.html). I used the closest weather station with a complete data set in each region. Temperature data for the Fort McMurray region were obtained from the Fort McMurray station (56° 39′ N 114° 13′ W), data for both Wabasca regions were from the Slave Lake station (55° 17′ N 114° 48′ W), and data for the Rocky-Drayton region were from the weather station at Rocky Mountain House (55° 22′ N, 114° 59′ W).

RESULTS

Measuring moth condition

M. disstria

All male *M. disstria* examined from sites at Rocky-Drayton were highly infected with microsporidia in 2006. All males examined from sites in Fort McMurray and both Wabasca regions were not infected. Therefore, I did not perform further analysis of microsporidia infection for *M. disstria*.

Geographical region and collection date (9 July and 1 August 2006) both had strong effects on *M. disstria* wing area (Fig. 3.1). Wing area decreased between 9 July and 1 August 2006 in all four regions (Fig. 3.1). Collection date had strong effect on wing area ($F_{2,166} = 42.50$, $P < 0.001$) when moths collected on 9 July, 1 August, and 19 August, 2006 were included in a separate analysis of the Fort McMurray region but area did not decrease further after 1 August (Tukey HSD; $P = 0.76$) (Fig. 3.1). Wing areas of males collected at the Wabasca-high region were smaller than all three other regions (Fig. 3.1). Traps at the Fort McMurray region attracted males with the largest wing area on 9 July 2006 but Fort McMurray males were smaller than both Rocky-Drayton and Wabasca-low males on 1 August (Fig. 3.1) as demonstrated by a significant interaction effect between area and date.

C. conflictana

Neither collection date nor microsporidia infection level affected wing area of male *C. conflictana* in either 2005 (Fig. 3.2A) or 2006 (Fig. 3.3A). Collection date did not affect microsporidia infection level in 2005 (Fig. 3.2B) or 2006 (Fig. 3.3B).

Flight phenology

M. disstria

In 2005, more degree-days (DD) had accumulated at each collection date in the Wabasca regions than the Rocky-Drayton region (Fig 3.4A). Trap capture of male *M. disstria* peaked at 664 DD in the Rocky-Drayton region, 736 DD in the Wabasca-high region and at 864 DD in the Wabasca-low region (Fig. 3.4A). Male *M. disstria* captures peaked on 21 July 2005 for Rocky-Drayton and Wabasca-high males, whereas Wabasca-low male captures peaked on 3 August 2005 (Fig 3.4A). The Wabasca-high region showed a distinct peak in numbers of males captured in traps in 2005 relative to other areas (Fig. 3.4A).

In 2006, the Fort McMurray region was the warmest with the greatest number of degree-days accumulated at each collection date followed by the Wabasca regions and then Rocky-Drayton (Fig. 3.5B). *M. disstria* trap capture peaked at 631 and 727 DD in the Rocky-Drayton and Wabasca-low regions respectively (Fig. 3.4B). Peak trap capture also occurred at the Wabasca-high region after 727 DD, and after 1079 DD in the Fort McMurray region (Fig. 3.4B). Male captures increased abruptly on 8 July 2006 and then

peaked on 31 July 2006 at the Fort McMurray region whereas captures in all three other regions peaked on 11 July 2006 (Fig. 3.4B).

C. conflictana

In 2005, capture of male *C. conflictana* peaked at both Wabasca regions after 566 DD and at the Rocky-Drayton region after 517 DD (Fig 3.5A). Peak trap capture occurred in all regions on 5 July 2005 with a notable increase in the number of males captures at the Wabasca-low region compared to the previous collection date (Fig. 3.5A).

In 2006, *C. conflictana* captures peaked at the Wabasca-high and Fort McMurray regions on 21 June 2006 after 493 and 593 DD, respectively had accumulated (Fig. 3.5B). Captures at the Fort McMurray region on 21 June 2006 were much higher than any other region (Fig. 3.5B). Male captures peaked after 631 and 727 DD at the Rocky-Drayton and Wabasca-low regions on 8 July 2006 (Fig. 3.5B).

DISCUSSION

This brief ecological study demonstrated the usefulness of pheromone trapping beyond the domain of IPM. Provided a non-saturating trap type is employed, this approach allows for the examination of morphological characters and pathological infections of important insect pest species, information that may be useful for studying population dynamics of important forest defoliators. My results show that pheromone trapping is a useful tool to detect both the presence and level of pathogen infection in forest Lepidoptera. I found high levels of microsporidia infection in all *M. disstria* males

examined on both collection dates from the Rocky-Drayton region in 2006. Conversely, spores were not found in any other region, at any collection date in male *M. disstria*. I expected to capture highly-infected male *M. disstria* at the Wabasca-high region because population density has been at the outbreak stage since 2001 and microsporidia infection levels are thought to increase with insect density (Thomson, 1958b; Wilson, 1977b, Fitzgerald, 1995). Spores will accumulate in the environment from silk or feces, or from disintegrating larval carcasses, which increases the probability of horizontal transmission (Maddox *et al.*, 1998). In 2005 and 2006, microsporidia were found in *C. conflictana* at all sites in the Rocky-Drayton region throughout the flight period at varying levels of infection. These results show that incidence and level of microsporidia infection varies among regions for populations of *M. disstria* and within and among regions for *C. conflictana*.

My findings are in accord with previous studies that showed the incidence of infection varies greatly among sites for *C. conflictana* (Wilson & Burke, 1971; Burke & Percy, 1982), *Malacosoma americanum* Fabricius (Lepidoptera: Lasiocampidae) (Nordin, 1976), *Choristoneura fumiferana* (Clem.) (Wilson, 1977b) and *Ostrinia nubilalis* Hübner (Hill & Gary, 1979). Several of these studies used larval samples and did not report infection rates by sex whereas my study examined adult males exclusively. Most species of microsporidia are transmitted horizontally and vertically (Maddox *et al.*, 1998), therefore both sexes are equally likely to be infected. Spores are found in all life stages of the insect but infection level may be highest in the adult stage due to ingestion of spores throughout the larval stages. Therefore, examination of adults provides a reliable method

to detect infection that would otherwise go undetected if only early instar larvae were examined. However, microsporidia infections are known to cause increased larval and pupal mortality in forest Lepidoptera (Wilson, 1977a; Bauer & Nordin, 1989), thus pheromone trapping may not be an appropriate method of sampling absolute infection rates in a population. Regardless, the objective of my study was to use pheromone trapping to assess adult male moth condition. It would be of interest in future studies to sample larval and pupal stages of *M. disstria* and *C. conflictana* to compare microsporidia infection levels with pheromone-trapped adult males.

Microsporidia infection level was not affected by flight period for either species contrary to my prediction. I expected to see an increase in infection level of both species over the duration of the flight period due to slower development of heavily infected individuals as has been observed with *C. fumiferana* (Thomson, 1958a; Wilson, 1977b; Eveleigh *et al.*, 2007). If adults that fly later in the season are a result of slower growing larvae in a population, then longer periods of foliage consumption will result in increased exposure to spores in the environment (Wilson, 1977b). Alternately, as microsporidia infections slow larval growth in both *M. disstria* (Wilson, 1977a) and *C. fumiferana* (Thomson, 1958b) moths that fly later in the season could be expected to be already infected. However, in the current study, *M. disstria* males in the Rocky-Drayton region were all highly-infected throughout the season. *M. disstria* males in this region were captured at sites in the vicinity (40-50 km) of a recently collapsed infestation near Rocky Mountain House. The collapsed infestation may have been a source of highly-infected females that dispersed and oviposited in the area where my sites were located. This hypothesis is

supported from examination of trap-captured *M. disstria* males at sites farther north in the Rocky-Drayton region (~ 100 km from collapsed infestation) that showed lower levels of infection (Meldrum, 2007). *C. conflictana* infection level varied by site and collection date without any obvious trend. Variation in infection level of captured male *C. conflictana* indicates that a proportion of highly-infected males develop and fly as well as lesser or uninfected males. Similarly, microsporidia infection in *C. fumiferana* did not affect flight duration or response to calling females in the laboratory (Sanders & Wilson, 1990). Larval mortality and reduced flight capability due to microsporidian infection may eliminate a proportion of highly-infected males from capture in traps for both *M. disstria* and *C. conflictana.*

I did not find any evidence that microsporidia infection affected wing size of either *M. disstria* or *C. conflictana* males. *M. disstria* males from the Rocky-Drayton region were all highly-infected and no infection occurred in moths sampled from the other regions. Therefore region and infection are confounded and it is not possible to conclude if infection would reduce wing size. However, the heavily infected *M. disstria* males from the Rocky-Drayton region had the second largest mean wing size among all four regions on 9 July 2006 and the largest on 11 July 2006 suggesting that infection alone does not influence moth size. Sanders & Wilson (1990) showed that *C. fumiferana* females infected with a "low" dose of microsporidian spores in the laboratory had smaller wings than uninfected females. In my study, *C. conflictana* males were from natural populations and consequently infection level was not controlled. Thomson (1958b) suggested that female *C. fumiferana* larvae artificially inoculated in the laboratory developed heavier

infections than those naturally infected in the field. In the current study, microsporidian infection did not influence male moth wing area in either species. This may be due to a bias in the sampling technique that reduced the number of highly-infected males that were captured due to reduced flight capability or pheromone responsiveness. Alternatively, the most highly-infected individuals may not survive to eclose as adults and are therefore not represented in the pheromone trap catch. This hypothesis is supported by work on monarch butterflies, *Danaus plexippus* L. (Lepidoptera: Nymphalidae), infected by a protozoan parasite, *Ophryocystis elektroscirrha* McLaughlin and Myers (Apicomplexa: Neogregarinida). Highly-infected *D. plexippus* had smaller wing areas than uninfected individuals (Altizer & Oberhauser, 1999) while low to moderate levels of infection did not affect wing area (Bradley & Altizer, 2005).

Trap-captured *M. disstria* males from the Wabasca-high region had significantly smaller wings than males from other regions on both collection dates in 2006. High population density can reduce individual body size (Elkinton & Liebhold, 1990) and wing length (Carter *et al.*, 1991) in *Lymantria dispar* L. Klemola *et al.* (2004) show that body size of the autumnal moth, *Epirrita autumnata* (Borkhausen) (Lepidoptera: Geometridae), at high population densities is smaller than when population densities are low. Possible causes of reduced body size at high defoliator densities are a quantitative shortage of foliage, short-term and delayed induced chemical defenses of the host tree, and larval crowding effects (Klemola *et al.*, 2004). In 2006, the Wabasca-high outbreak was in its fifth year, thus *M. disstria* males captured may be smaller for all of these reasons. Males captured at sites in the Fort McMurray region had the highest wing area on 9 July 2006

but the second lowest on 1 August 2006. The significant interaction between date and region on male wing size may be due to migration of smaller individuals from the Wabasca-high outbreak before the end of the flight season. The Fort McMurray region is approximately 150 km east of the Wabasca-high sites. Although *M. disstria* adult dispersal ability is poorly documented, there is some evidence that a migration of this magnitude is possible (Fitzgerald, 1995).

Both male *M. disstria* and *C. conflictana* wing area decreased during the course of the flight season. Aspen foliage quality declines over the course of the growing season through the increase of plant defensive compounds including phenolic glycosides and condensed tannins, as well as constitutive changes such as increased leaf toughness, and decreased nitrogen and water content (Hunter & Lechowicz, 1992; Osier *et al.*, 2000). In addition, short-term induced resistance from herbivory would change foliage chemistry (Clausen *et al.*, 1989). All of these factors are suggested to adversely affect development of *M. disstria* larvae (Hemming & Lindroth, 1999; Kopper & Lindroth, 2003). *C. conflictana* larvae would face similar challenges when developing on aspen foliage (Bryant *et al.*, 1987; Clausen *et al.*, 1989). Larvae that hatch late or grow slowly will obtain poorer quality food than those that hatch early or grow fast (Witter & Waisanen, 1978; Hunter & Lechowicz, 1992; Jones & Despland, 2006). Adult size is determined by larval size in insects, thus adults that eclose later in the season are usually smaller than those that eclose earlier (Eveleigh *et al.*, 2007).

Pheromone-baited traps proved to be an excellent method for determining the flight phenology of *M. disstria* and *C. conflictana* males. There were adequate data to establish the flight period for each species despite two-week intervals between collection dates. The *M. disstria* phenology established in the current study closely resembles that of a previous study based upon collection records for Alberta from 1915-2000 (Schmidt & Roland, 2003). The fact that there was little difference between numbers of male *M. disstria* captured in high and low density regions is a function of pheromone-response at high population densities (Chapter 2).

I was unable to tease apart the effect of temperature on flight phenology due to the confounding effect of population density among regions. Although 2006 was a warmer year and male *M. disstria* were captured earlier in June than in 2005, those captures only occurred at sites in the Wabasca-high and Fort McMurray regions where population densities were above endemic levels. The Rocky-Drayton and Wabasca-low regions are more directly comparable as both regions have endemic populations of *M. disstria* with similar trap catches. Within 2005, male captures peaked earlier at Rocky-Drayton sites although temperatures were cooler than at Wabasca-low sites. This phenomenon may be due to low captures in both regions so male flight may not be detectable by pheromone trapping. There is also better resolution to detect flight in the Rocky-Drayton region (n=9) than in the Wabasca-low region (n=5).

The effect of population density on *M. disstria* flight phenology is demonstrated through a large increase in catch at Wabasca-high sites during mid-July of each year. A greater

proportion of males flew earlier in the season at high-density sites as compared to low-density sites. High-density populations of *L. dispar* develop more quickly than low-density populations and adults fly earlier (Elkinton & Liebhold, 1990). Quicker development may be due to increased larval body temperature because high-density populations remain in the canopy all day instead of seeking shelter, and defoliation exposes larvae to greater solar radiation (Elkinton & Liebhold, 1990). These factors could be equally applicable to high-density populations of *M. disstria*. In 2006, traps at sites in the Fort McMurray region captured more male *M. disstria* than traps in any other region and flight did not peak until late July. This may be a function of migration of moths from the Wabasca-high region. Trap catch at sites in Fort McMurray were generally higher than expected for an endemic population level which indicates that male captures in pheromone-baited traps detected an increase in the resident population.

Flight periods were similar among regions for *C. conflictana* in 2005 and there was no obvious effect of population density. However, *M. disstria* population density may have affected *C. conflictana* flight in 2006. There was a large increase in *C. conflictana* captures on 21 June, 2006 in the Fort McMurray region and relatively few males were captured later in the season. This pattern may be the effect of an increasing *M. disstria* population in the Fort McMurray region. Ives and Wong (1988) reported that *C. conflictana* outbreaks precede *M. disstria* outbreaks. The high number of *C. conflictana* captures at the Fort McMurray sites may represent the end of a higher density population before the increase in *M. disstria* density detected in this study. This interaction may be the result of *M. disstria* out-competing *C. conflictana* to force a phenological shift in

flight time. The pattern would not be observed in the Wabasca-high region because the *M. disstria* outbreak is five-years old and *C. conflictana* only exist at low abundance in this region. In 2006, the *C. conflictana* flight period was truncated compared to 2005 possibly because temperatures were warmer in 2006 and insects developed more quickly.

The data I present here support the use of pheromone trapping to assess condition of adult male *M. disstria* and *C. conflictana*. I demonstrated that pheromone-baited traps used in an IPM program could also provide data on wing area and pathogen infection in addition to flight phenologies for each species. Indicators of adult male condition may be used to make inferences about population quality and thus population dynamics. Although it appears that sublethal infections of the protozoan parasite microsporidia may have little effect on adult condition, wing area is a suitable indicator of moth condition. Future work should involve analyses of other wing dimensions such as length and width. Wing length has been shown to be a possible predictive factor for monitoring *L. dispar* populations (Bellinger *et al.*, 1990; Carter *et al.*, 1991). Although pheromone trapping of *L. dispar* is not a suitable technique for identifying temporal patterns in adult flight (Carter *et. al.*, 1992), the data presented here indicate otherwise for *M. disstria* and *C. conflictana*. I propose that pheromone trapping has ecological considerations that are undervalued within the context of IPM.

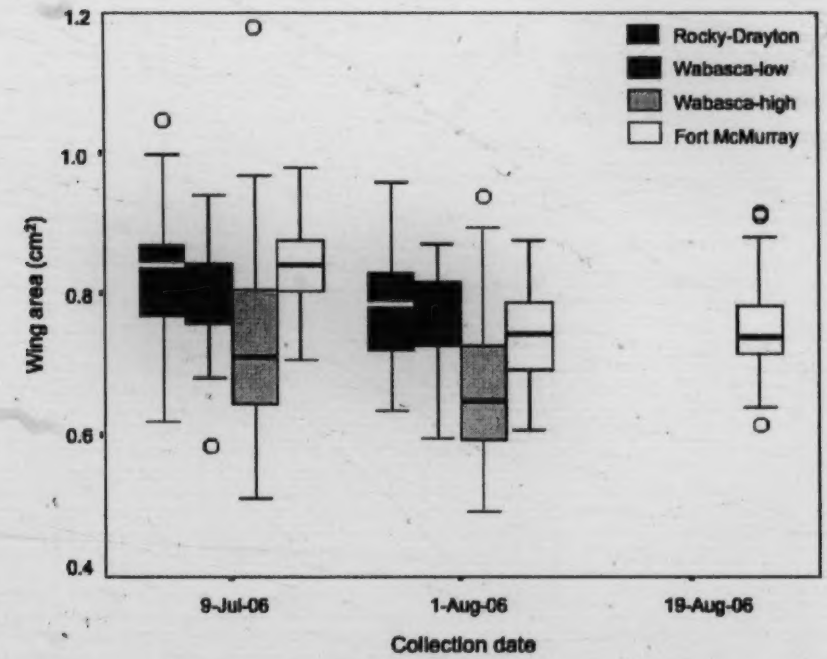


Figure 3.1. Effect of collection date ($F_{1,419} = 54.35$, $P < 0.001$) and geographical region ($F_{3,419} = 36.10$, $P < 0.001$) with an interaction ($F_{3,419} = 3.34$, $P = 0.011$) on mean ± SE right forewing area of *M. disstria* in 2006. Wing area for Wabasca high-density region males differ from all other regions (Tukey HSD; $P < 0.001$). Mean wing area for males captured on 19 August 2006 from Fort McMurray not included in analysis. The horizontal line shows the median with the box representing the interquartile range. Whiskers show 1.5 times the interquartile range with outliers beyond this range indicated by open circles.

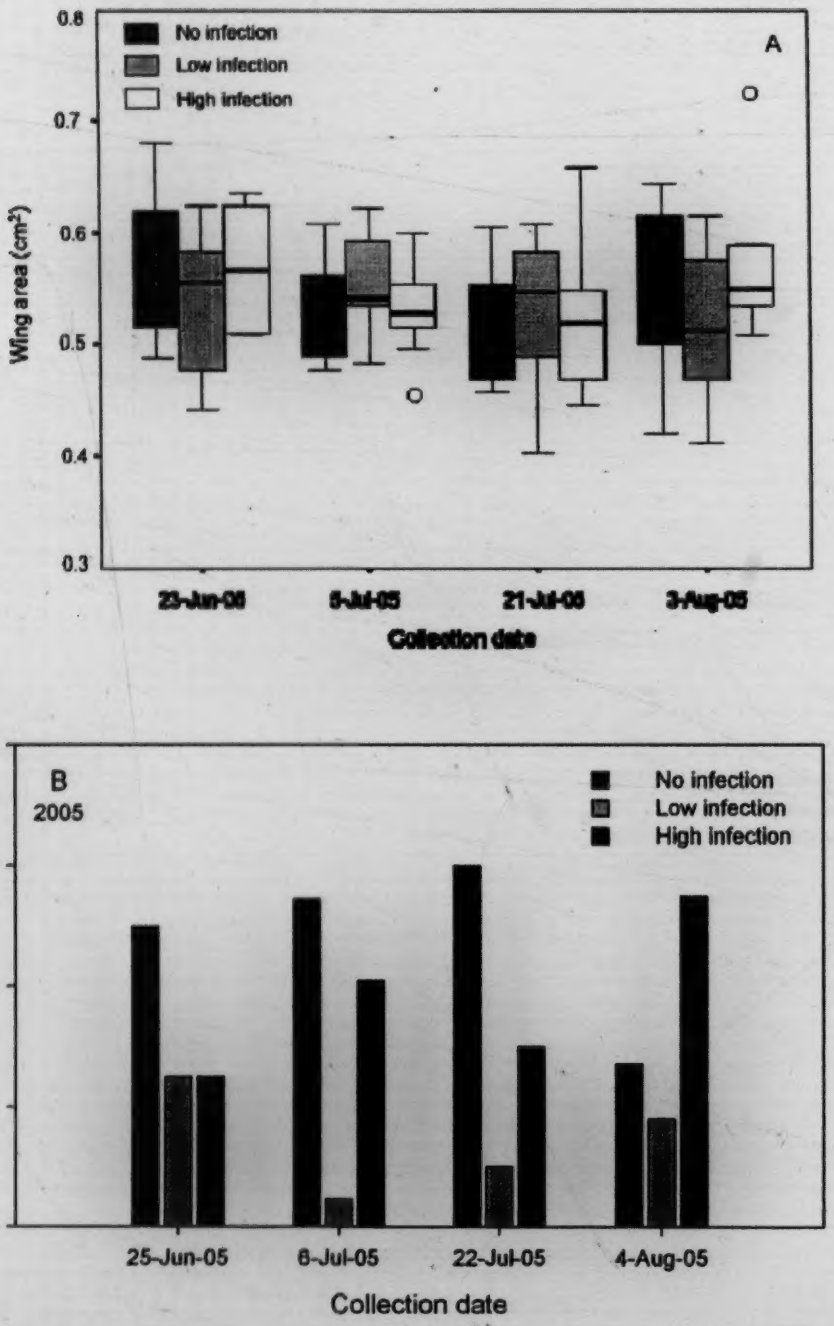
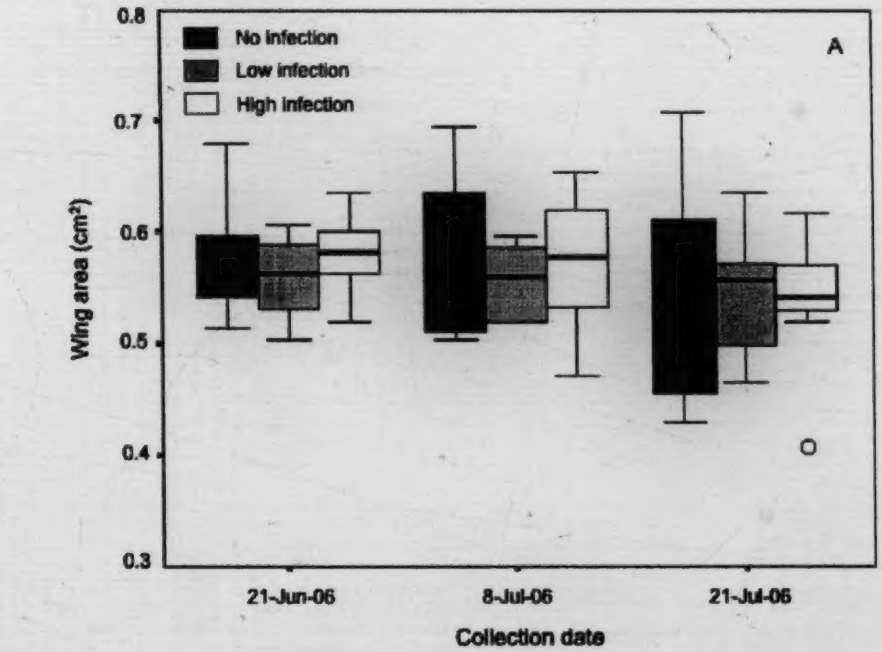


Figure 3.2. (A) Effect of collection date ($F_{3,105} = 1.41$, $P = 0.25$) and microsporidia infection level ($F_{2,105} = 0.36$, $P = 0.70$) with no interaction ($F_{6,105} = 1.05$, $P = 0.40$) on mean ± SE right forewing area of male *C. conflictana* in 2005. (B) Effect of collection date on microsporidia infection level ($\chi^2_6 = 10.17$, $P = 0.12$). Plots as defined in Fig. 3.1.



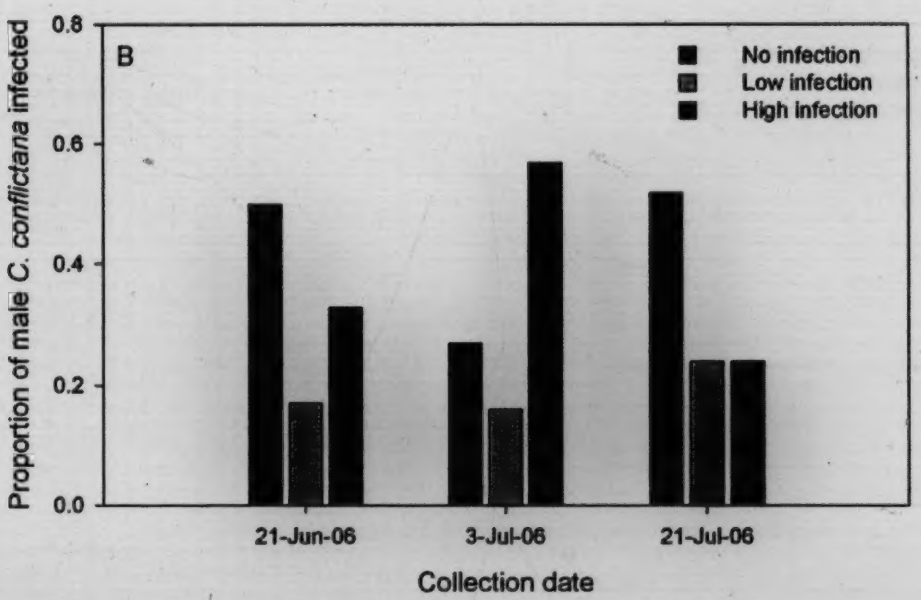


Figure 3.3. (A) Effect of collection date ($F_{2,93} = 1.32$, $P = 0.27$) and microsporidia infection level ($F_{2,93} = 0.98$, $P = 0.38$) with no interaction ($F_{4,93} = 0.64$, $P = 0.63$) on mean ± SE right forewing area of male *C. conflictana* in 2006. (B) Effect of collection date on microsporidia infection level ($\chi^2_4 = 8.85$, $P = 0.065$). Plots as defined in Fig. 3.1.

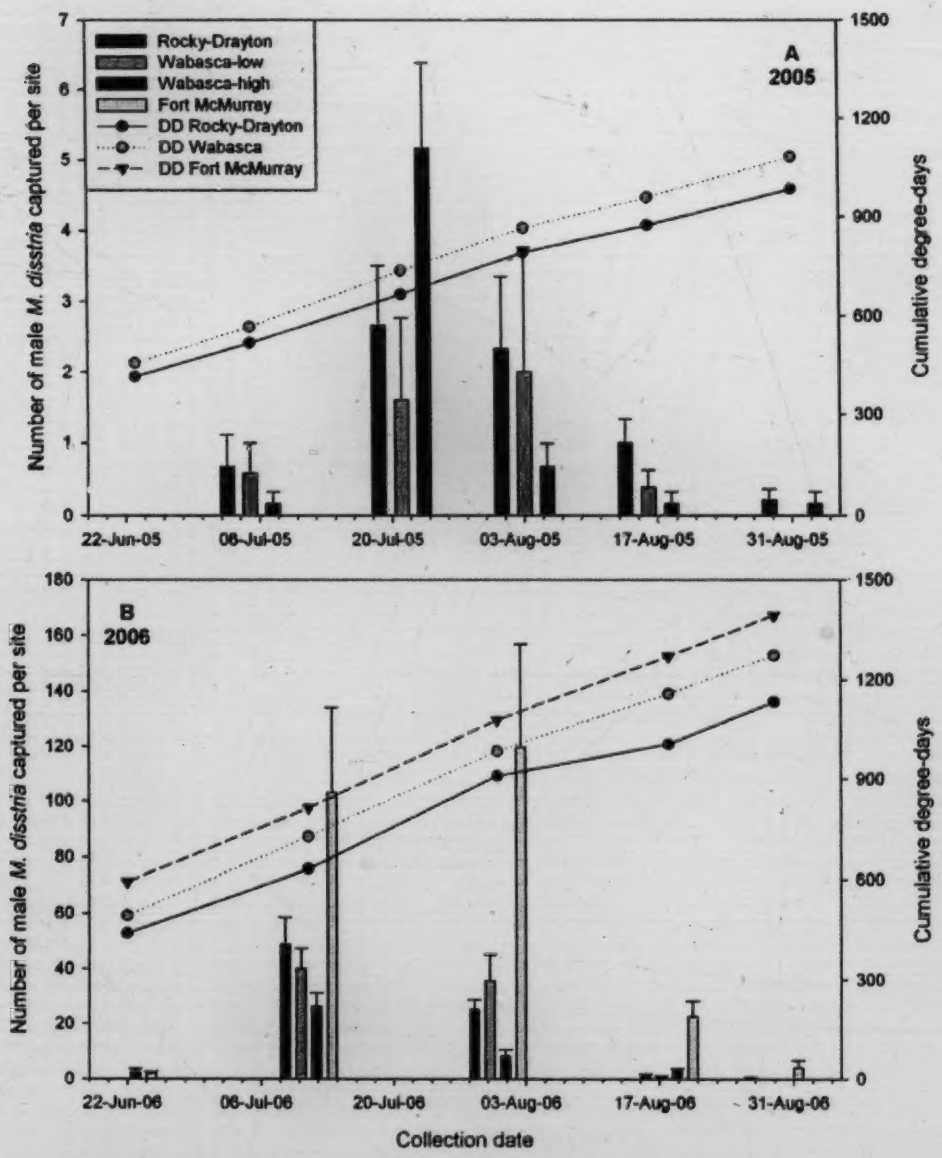


Figure 3.4. (A) Flight phenology for *M. disstria* in 2005 and (B) 2006 based on mean ± SE male adult captures per site in combined pheromone-baited traps. A collection date represents a 3-day interval when moths were collected from all regions. There were 9, 5, 6, and 5 sites in the Rocky-Drayton, Wabasca-low, Wabasca-high and Fort McMurray regions respectively. Fort McMurray sites Cumulative degree-days were calculated with temperature data from proximal weather stations.

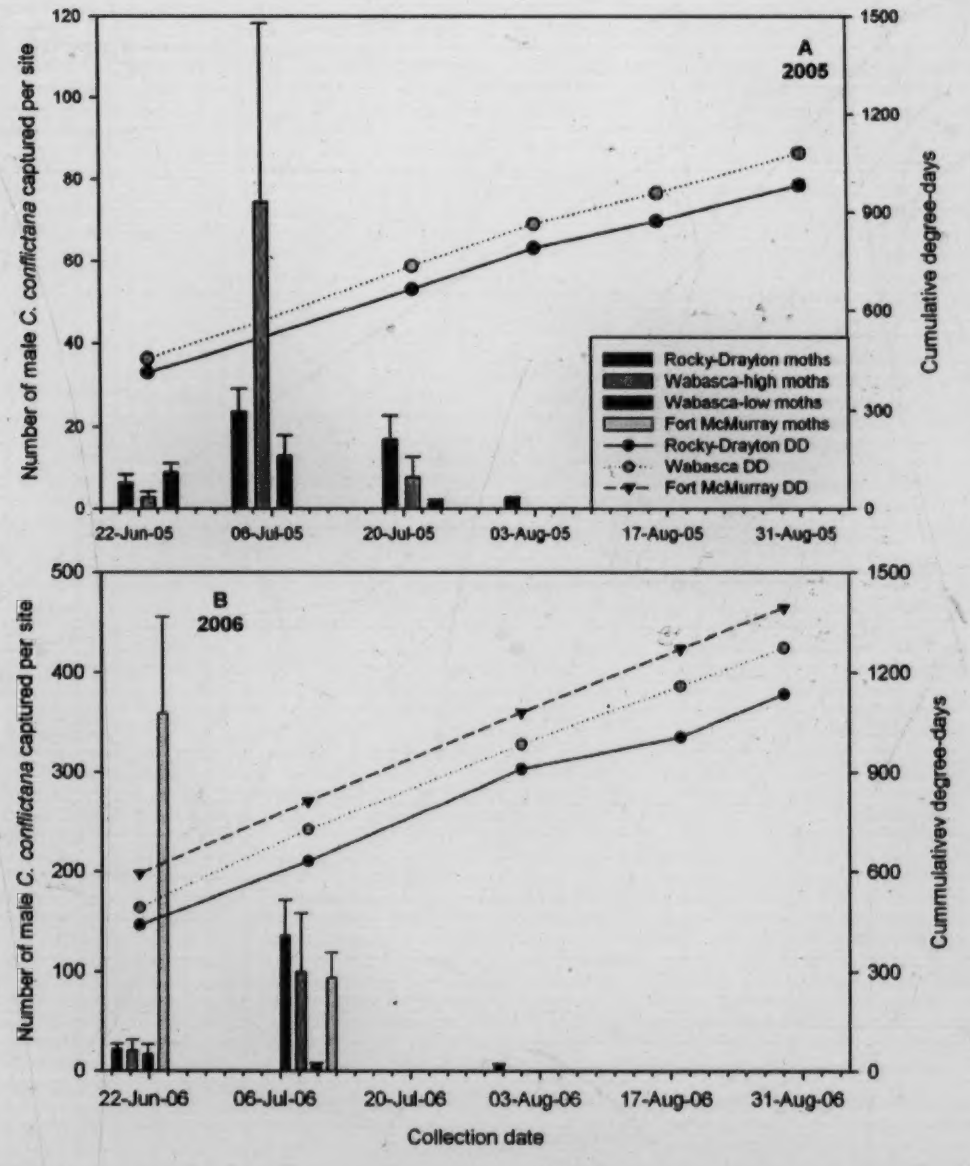


Figure 3.5. (A) Flight phenology for *C. conflictana* in 2005 and (B) 2006 based on male adult captures per site in combined pheromone-baited traps. A collection date represents a 3-day interval when moths were collected from all regions. There were 9, 5, 6, and 5 sites at the Rocky-Drayton, Wabasca-low, Wabasca-high and Fort McMurray regions respectively. Cumulative degree-days were calculated with temperature data from proximal weather stations.

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Chapter 4

General Discussion and Conclusions

Sex pheromone-based monitoring is a species-specific tool and the combination of two species' pheromone together in one lure represents a novel approach. Adult male Lepidoptera captured by the monitoring system must be related to other estimates of population density in order to predict incipient outbreaks. The goal of this thesis was to develop a pheromone-based monitoring system for two sympatric aspen defoliators in Alberta: *Malacosoma disstria* Hübner and *Choristoneura conflictana* (Walker). In addition, I demonstrated that insects captured in traps have value for ecological studies that can compliment pest management initiatives.

A combined pheromone-based monitoring tool

My research demonstrates that a combined approach to monitoring populations of *M. disstria* and *C. conflictana* with one pheromone blend, one lure and one trap is feasible. The combined lure was as attractive to both *M. disstria* and *C. conflictana* males as traps baited with each species' pheromone alone (Chapter 2). However, two anomalous results were recorded. I found that all traps placed in stands where *M. disstria* was at outbreak densities captured equal numbers of males regardless of pheromone treatment. This was most likely due to the great number of male *M. disstria* in flight and the great probability they would encounter a trap. I also found reduced captures of *M. disstria* at sites within the outbreak when compared with captures from other sites at lower population densities. Reduction in catch may have been due to the "competition effect" (Cardé, 1979), a lack of visual cues for male moths, larval parasitism over-estimating adult abundance, or a synthetic pheromone that was less attractive than calling females (Chapter 2). Unfortunately, the high-density population of *C. conflictana* targeted in this study

collapsed in 2005, and therefore I could not compare density-dependent results between species. The lure longevity experiment showed that lure age did not affect capture of *C. conflictana* males but *M. disstria* was less attracted to the combined lures greater than five weeks old (Chapter 2). The release rate of pheromone from rubber septa decreases exponentially with time (Butler & McDonough, 1981) thus future development of the system will require larger amounts of pheromone to be tested over time.

Trap captures of *M. disstria* and *C. conflictana* males were related to immature samples both within and between generations (Chapter 2). Although larval counts were strongly related to captures of male *C. conflictana*, male captures did not predict larval counts in the next generation. Without a high-density population of *C. conflictana* available to provide a breadth of densities, it was difficult to establish the predictive model for this species. I was more successful with *M. disstria* although male captures did not always predict immature numbers in the next generation. Pupal counts appear to be the best indicator of *M. disstria* population density and were indicative of severe defoliation (Chapter 2). Thus an opportunity exists for further research to examine this relationship more closely and determine whether the combined lure could predict defoliation. I discovered a curvilinear relationship between male *M. disstria* captures and pupal counts whereby numbers of male moths captured in pheromone-baited traps level off at high densities as measured by pupal counts (Chapter 2). This phenomenon has been previously demonstrated when *M. disstria* density is estimated by pupal counts (Roland, 2005) or by defoliation (Schmidt *et al.*, 2003).

Moth condition

Pheromone-based monitoring programs provide an excellent opportunity to collect male moths for ecological studies. In Chapter 3, I demonstrated that moth condition could be measured using males collected in pheromone traps. My results showed that pheromone trapping provided a method to track microsporidian infections in populations of *M. disstria* and *C. conflictana*. I determined presence/absence of infection for both moth species but found no discernable trends for the effects of flight season or population density on infection level. Although there was no direct comparison available in the literature, previous work on *C. conflictana* indicated microsporidian infection varied among sites (Wilson & Burke, 1971; Burke & Percy, 1982). I did not capture enough moths to test for among-site differences in moth infection level but future work could explore this avenue. Moth wing area was affected by flight period and population density only for *M. disstria* (Chapter 3). This result underlines the utility of the pheromone trapping method to show that the effect of flight period on wing area differs between species. In Chapter 3, I also demonstrated that the flight phenology of *M. disstria* and *C. conflictana* could be established using pheromone traps. The flight period established for *M. disstria* in 2006 was similar to that shown by Schmidt & Roland (2003) based on historical records.

Summary

To the best of my knowledge, moths captured in pheromone-baited traps are rarely examined for factors that may indicate population condition. Pheromone trapping has been used in ecological studies with respect to population dynamics (Roland, 2005),

population distribution (Meagher & Nagoshi, 2004), and dispersal (Schneider, 1999). However, the same arguments used against pheromone trapping in IPM programs can be used for these ecological studies. That is, whether captured males are representative of the local, breeding population (McNeil, 1991). One recent study of *Thaumetopoea pityocampa* Denis & Schiffermüller in Italy confirmed that this is indeed the case for core populations although not necessarily so when trapping at the edge of the distribution range (Salvato *et al.*, 2005). Therefore, this thesis is further support of pheromone trapping for both monitoring of insect pest species and ecological studies based on those captured species.

Ecological data collected from examination of moths captured in pheromone-baited traps may also provide useful information for pest management. My results showed an increase in *M. disstria* trap catch at the Fort McMurray region late in the flight season potentially due to emigration from the Wabasca-high region. Further work is needed to support this hypothesis but high numbers of captures at Fort McMurray may be evidence of *M. disstria* dispersal from the outbreak area. *M. disstria* moths are likely capable of dispersing (Fitzgerald, 1995) as are other forest Lepidoptera such as *Choristoneura fumiferana* (Clem.) (Greenbank *et al.*, 1980; Nealis & Régnière, 2004). I did not relate wing area measured in Chapter 3 with density estimates of either species in Chapter 2;a however, Bellinger *et al.*, (1990) used pheromone traps to capture *Lymantria dispar* L. males and successfully correlate wing length with moths per trap and eggs per mass in the subsequent generation. The biological basis of this relationship is poorly understood

but implicates moth condition as a potential predictive variable in pheromone-based monitoring programs.

This thesis demonstrates the potential to develop a combined sex pheromone-based system for *M. disstria* and *C. conflictana*. Combining the pheromone of two distantly related species into one lure represents a novel approach. Although Evenden (2005) demonstrated the efficacy of this combined lure with endemic populations of each species, my work targeted a breadth of population densities and tested the lure's longevity and predictive ability. The combined tool represents an important contribution to forest pest management due to anticipation of intensive aspen silviculture. Available forest for harvest will be reduced in the future due to the demands of conservation and use in other anthropogenic activities. Thus controlling insect defoliation that reduces tree growth and yield of aspen stands will be of paramount concern.

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Appendix A

APPENDIX A.
Detailed site descriptions

Region ***Site***	**Geographic coordinates**	**Height (m)** [1,2] **Dbh (m)** [1,2]	**Natural region**[3] ***Subregion***[3]	**Most abundant canopy species per study region**	**Most abundant understory species per study region**
Rocky- Drayton (north)					
RD1	53□12«29««N 114□45«32««W	12.09 ± 1.19 0.124 ± 0.021	Boreal forest *Central/Dry mixedwood*	Trembling aspen (*Populus tremuloides)* Balsam poplar (*Populus balsamifera)* White spruce (*Picea glauca)*	Prickly rose (*Rosa acicularis*) Low bush cranberry (*Viburnum edule*) Willow (*Salix* spp.) Beaked hazelnut (*Corylus cornuta*)
RD2	53□12«09««N 114□36«14««W	12.41 ± 2.06 0.108 ± 0.013			
RD3	53□09«49««N 114□53«44««W	13.9 ± 2.49 0.122 ± 0.018		White birch (*Betula papyrifera*	
RD4	53□07«06««N 114□51«24««W	11.11 ± 1.91 0.107 ± 0.016			
Rocky-Drayton (south)					
RD6	52□40«30««N 114□49«25««W	12.58 ± 1.02 0.117 ± 0.012	Foothills *Lower foothills*	Trembling aspen (*Populus tremuloides)* Balsam poplar (*Populus balsamifera)* White spruce (*Picea glauca*)	Green alder (*Alnus viridis*) Common bearberry (*Arctostaphylos uva-ursi*) Low bush cranberry (*Viburnum edule*)

Region *Site*	Geographic coordinates	Height (m) [1,2] Dbh (m)[1,2]	Natural region[3] *Subregion*[3]	Most abundant canopy species per study region	Most abundant understory species per study region
RD8	52□38«07««N 114□53«43««W	16.46 ± 1.21 0.148 ± 0.013			
RD9	52□34«52««N 114□54«18««W	14.03 ± 2.68 0.122 ± 0.023			
RD10	52□36«01««N 114□54«56««W	15.22 ± 1.39 0.129 ± 0.025			
Wabasca-low					
WL1	55□30«23««N 113□27«33««W	14.53 ± 0.40 0.135 ± 0.005	Boreal forest *Central mixedwood*	Trembling aspen (*Populus tremuloides*) Balsam poplar (*Populus balsamifera*) White spruce (*Picea glauca*) White birch (*Betula papyrifera*) Balsam fir (*Abies balsamea*)	Green alder (*Alnus viridis*) Prickly rose (*Rosa acicularis*) Low bush cranberry (*Viburnum edule*) Wild red raspberry (*Rubus idaeus*) Bunchberry (*Cornus canadensis*)
WL2	55□41«13««N 113□23«49««W	14.15 ± 2.25 0.152 ± 0.030			
WL3	55□42«49««N 113□24«07««W	13.11 ± 1.17 0.123 ± 0.015			
WL4	55□44«13««N 113□24«14««W	13.92 + 1.12 0.124 ± 0.016			
WL5	55□45«11««N 113□24«28««W	17.16 ± 1.81 0.145 ± 0.018			

Region *Site*	Geographic coordinates	Height (m) [1,2] Dbh (m) [1,2]	Natural region [3] *Subregion* [3]	Most abundant canopy species per study region	Most abundant understory species per study region
Wabasca-high					
WH6	56□15«53««N 113□49«23««W	15.15 ± 0.88 0.128 ± 0.006	Boreal forest *Central mixedwood*	Trembling aspen (*Populus tremuloides*) Balsam poplar (*Populus balsamifera*) White spruce (*Picea glauca*) White birch (*Betula papyrifera*) Balsam fir (*Abies balsamea*)	Green alder (*Alnus viridis*) Prickly rose (*Rosa acicularis*) Low bush cranberry (*Viburnum edule*) Wild red raspberry (*Rubus idaeus*) Bunchberry (*Cornus canadensis*)
WH7	56□16«39««N 113□49«18««W	15.09 ± 1.42 0.140 ± 0.037			
WH8	56□17«12««N 113□49«49««W	17.52 ± 1.59 0.133 ± 0.020			
WH9	56□16«42««N 114□44«02««W	15.32 ± 2.24 0.135 ± 0.021			
WH10	56□17«31««N 113□43«54««W	14.64 ± 1.53 0.129 ± 0.018			
WH11	56□18«18««N 113□42«19««W	14.80 ± 1.71 0.129 ± 0.016			
WH12	56□18«47««N 113□41«59««W	15.98 ± 1.51 0.131 ± 0.016			
WH14	56□20«01««N 113□41«05««W	15.16 ± 1.20 0.136 ± 0.023			
WH15	56□20«37««N 113□41«02««W	16.33 ± 2.51 0.128 ± 0.037			

Region *Site*	Geographic coordinates	Height (m) [1,2] Dbh (m) [1,2]	Natural region [3] *Subregion* [3]	Most abundant canopy species per study region	Most abundant understory species per study region
Fort McMurray					
FM1	56□11«02««N 111□43«39««W	n/a n/a	Boreal forest *Lower boreal highlands*	Trembling aspen (*Populus tremuloides)* Balsam poplar (*Populus balsamifera)* White spruce (*Picea glauca)* Lodgepole pine (*Pinus contorta)* Jack pine (*Pinus banksiana)*	Red-osier dogwood (*Cornus stolonifera*) Common labrador tea (*Ledum groenlandicum)* Green alder (*Alnus viridis*) Willow (*Salix* spp.)
FM2	56□13«22««N 111□41«35««W	n/a n/a			
FM3	56□15«14««N 111□37«58««W	n/a n/a			
FM4	56□17«54««N 111□35«37««W	n/a n/a			
FM5	56□20«39««N 111□35«02««W	n/a n/a			

1 Š Mean ± SD of all felled trees at each site for 2005 and 2006
2 Š Data not available for Fort McMurray sites because no trees were cut
3 Š Natural Regions Committee. 2006. *Natural Regions and Subregions of Alberta*. Compiled by D.J. Downing and W.W. Pettapiece. Government of Alberta. Pub. No. T/852.